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Biomedical Applications of Layer-by-Layer Self-Assembly for Cell Encapsulation: Current Status and Future Perspectives

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Encapsulating living cells within multilayer functional shells is a crucial extension of cellular functions and a further development of cell surface engineering. In the last decade, cell encapsulation has been widely utilized in many cutting-edge biomedical fields. Compared with other techniques for cell encapsulation, layer-by-layer (LbL) self-assembly technology, due to the versatility and tunability to fabricate diverse multilayer shells with controllable compositions and structures, is considered as a promising approach for cell encapsulation. This review summarizes the state-of-the-art and potential future biomedical applications of LbL cell encapsulation. First of all, a brief introduction to the LbL self-assembly technique, including assembly mechanisms and technologies, is made. Next, different cell encapsulation strategies by LbL self-assembly techniques are explained. Then, the biomedical applications of LbL cell encapsulation in cell-based biosensors, cell transplantation, cell/molecule delivery, and tissue engineering, are highlighted. Finally, discussions on the current limitations and future perspectives of LbL cell encapsulation are also provided.

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1. Introduction

The past few decades have witnessed rapid development of encapsulating living cells within multilayer polymer shells for biomedical applications.[1,2] Cell encapsulation, which refers to the immobilization of viable cells within semipermeable and biocompatible polymeric membranes, has raised much attention in biomedicine, biotechnology, and bioelectronics fields.[3,4] The polymer shells could offer efficient transfer of oxygen, nutrients, and cell metabolite by-products while maintaining cell viability and functionality. Since the encapsulation shell could function as an immunoisolation barrier for the encapsulated cells and protect cells from harsh or hostile environment, cell encapsulation has been widely applied in many cutting-edge fields, such as cell-based transplantation therapy, drug delivery, tissue engineering, cell-based biosensor, etc.[5–7] Furthermore, cell encapsulation could offer a well-defined and isolated microenvironment mimicking the extracellular matrix of natural tissues, which is beneficial for the observation of cell–microenvironment interaction and modulation of cell function.[8,9]

Since the first literature on encapsulating islet cells within alginate bulk hydrogel beads in 1980,[10] considerable efforts have been devoted to encapsulating different cells with diverse materials and processes. Due to the ability to regulate cell encapsulation at the nanometer- or micrometer-scale, highly hydrated polymers cross-linked hydrogels which have a similar structure to the extracellular matrix of natural tissues have exhibited remarkable versatility in cell encapsulation and have once been considered as the standard process for cell encapsulation.[11] However, several intrinsic limitations of the use of bulk hydrogels, including limited stability, low permeability, and short sustenance period, have restricted their practical applications and raised the need to find alternative cell encapsulation techniques.[12,13] Some other techniques have been put forward to achieve cell encapsulation such as cell electrospinning,[14,15] bio-electrospraying,[16–18] aerodynamically assisted biojetting,[19,20] and biothreading.[21] These techniques exploit the electric field or air pressure as the driving force to pump the polymer solution containing cells through a needle to form cell-containing fibers, and have been widely utilized to build 3D cell-laden constructs. A great many relevant

pioneering works have been done and reviewed by Jayasinghe's group and their discoveries demonstrated the great potential of these techniques to be introduced into the clinic for many disease management in the future.[22,23] In recent years, layer-by-layer (LbL) self-assembly, as a simple but highly versatile multilayer self-assembly technique, has emerged as another promising alternative strategy for cell encapsulation. It has the capability to fabricate diverse multilayer shells with tunable compositions and structures to meet the needs of a variety of biomedical applications (as shown in **Figure 1**).

Owing to the rapid development of assembly technologies of LbL self-assembly technique, numerous LbL assembly strategies for cell encapsulation have been put forward for different cell species, including bacteria, yeasts, stem cells, erythrocytes, platelets, tumor cells, islet cells, etc., and have made remarkable achievements in attenuating host immune responses or deleterious environmental harm to transplanted cells, maintaining the metabolic activity and function of encapsulated cells, and modulating cell proliferation and differentiation.[24–32] In this review, we attempt to discuss the current status and future perspectives of the biomedical applications of LbL self-assembly technique for cell encapsulation. It will start with an introduction to the LbL self-assembly technique, including basic principles, assembly mechanisms, assembly materials, and assembly technologies. Then we focus on the cell encapsulation strategies of LbL self-assembly technique and summarize the biomedical applications of LbL cell encapsulation. Finally, we discuss the future perspectives of LbL self-assembly technique for cell encapsulation.

2. LbL Self-Assembly Technique

2.1. Basic Principles of the LbL Self-Assembly Technique

LbL self-assembly refers to an ultrathin film fabrication technique via sequentially depositing multilayers of oppositely charged materials, which was first introduced by Iler in 1966[33] and then received continuous development after the pioneering work of Decher in 1990s.[34,35] From then on, LbL assembly technique has been widely utilized as a versatile, convenient, and highly efficient strategy to fabricate multilayer materials with tunable structures, functions, and physicochemical properties.[25,36–39] Generally, the LbL self-assembly process involves the sequential exposure of a charged substrate to polycation and polyanion solutions to achieve adsorption of complementary materials on the substrate surface.[40–42] The adsorption steps can be repeated for several times to obtain the desired number of coating layers. The properties of the LbL self-assembly constructs can be modulated through adjusting some processing parameters such as coating material concentration, ionic strength of the medium, temperature, PH, washing and drying conditions.[43–46] An array of materials can be utilized as the assembly blocks for LbL assembly, including natural and synthetic polyelectrolytes, clays, peptides, metal oxides, enzymes, and polymer gels.[27,28,47,48] The LbL assembly technique could be utilized to encapsulate various substrates regardless of the shape and produce homogeneous nanocoatings with precise control of the structure. Besides, the whole assembly process can be performed under mild conditions.[49–51]

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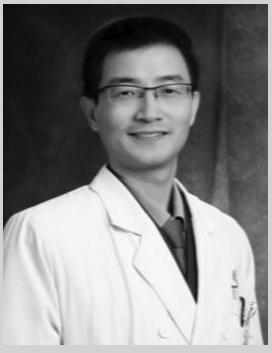

Gaoxing Luo received postdoctoral training in Yale University in USA. He is currently working as the director, professor, and surgeon in the Institute of Burn Research, Southwest Hospital, Army (Third Military) Medical University, China. He also serves as the vice chairman of the Chinese Burn Association. His research interests include the application of stem cells for tissue engineering and the development of interdisciplinary strategies for wound repair and regeneration

Malcolm Xing is a professor at the Faculty of Engineering and Medicine, the University of Manitoba, and Children's Hospital Research Institute of Manitoba, Canada. He completed his Ph.D. (2007) in biosystem engineering from UC Davis, and after received two years of postdoctoral fellow training in Brigham and Women's Hospital, Harvard. His interdisciplinary research team is focused on smart biomaterials for tissue engineering, nanomedicine, and wearable/implantable biosensors and biorobots. He is the recipient of Discovery Accelerator Supplements Award of National Science and Engineering Research Council of Canada, Canada Foundation for Innovation and Dr. John A. Moorhouse Diabetes Fellowship of Manitoba Diabetes Foundation.

2.2. Mechanisms of LbL Self-Assembly Technique for Cell Encapsulation

In general, the formation of LbL self-assembly multilayers is driven by multiple intermolecular interactions, including electrostatic interaction, covalent bonding, hydrogen bonding, van der Waals forces, hydrophobic interactions, charge-transfer

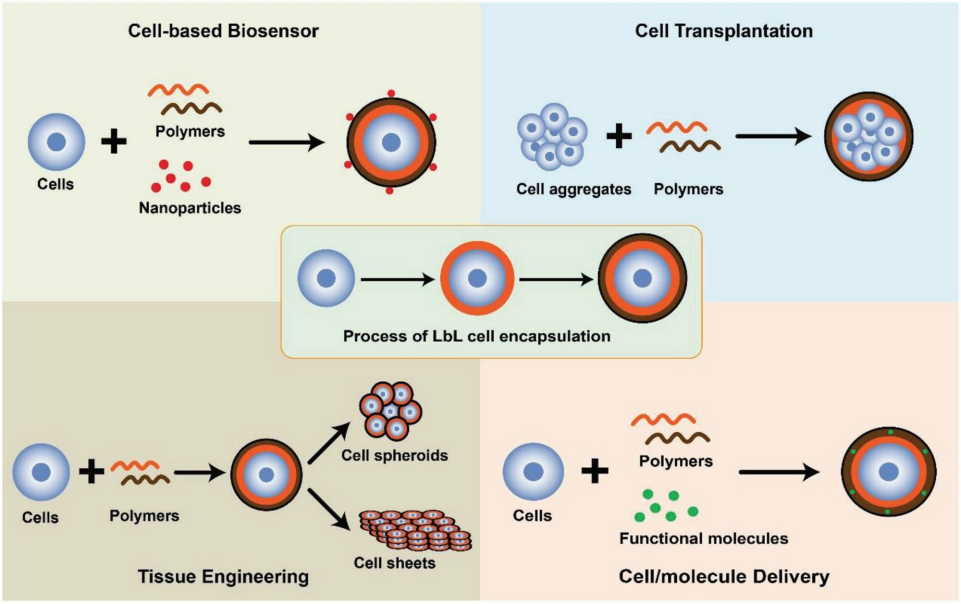

Figure 1. Schematic overview of the biomedical applications of LbL self-assembly technique for cell encapsulation and the process of cell encapsulation by LbL self-assembly technique.

interactions, host–guest interactions, etc.[52] Among these interactions, electrostatic interaction between the oppositely charged constituents is the most commonly applied driving force for the building up of nanostructured polyelectrolyte multilayers for cell encapsulation. Covalent bonding intermolecular interaction in LbL self-assembly multilayers for cell encapsulation is generally applied between the cell and the first layer.[53] The covalent conjugation of the polymer to the cell could also lead to the chemical or enzymatic modification of cell surface. Furthermore, the amphiphilic polymers that bear hydrophobic alkyl chains can anchor into the lipid bilayer cell membrane through the hydrophobic interactions.[47] Each intermolecular interaction has its merits and drawbacks. The optimization and combination of these interactions could significantly facilitate the fabrication of various LbL assembly materials for cell encapsulation to realize versatile applications.

2.3. Assembly Materials Used in the LbL Self-Assembly Technique for Cell Encapsulation

2.3.1. Polyelectrolytes

A plethora of materials have been utilized for the LbL encapsulation of cells.[27,48] Numerous polyelectrolytes, according to their origin, can be generally divided into two parts: synthetic polyelectrolytes and natural polyelectrolytes. The most commonly used synthetic polyelectrolytes include poly(styrene sulfonate) (PSS), poly(allylamine) (PAH), poly-L-lysine (PLL), poly (dimethyldiallylammonium chloride) (PDDA), poly(ethylenimine) (PEI), poly(N-isopropyl acrylamide (PNIPAM), poly(acrylic acid) (PAA), poly (methacrylic acid) (PMA), and poly(vinyl sulfate) (PVS). Natural polyelectrolytes include proteins (gelatin, lysozymes, and albumin), nucleic acids, and polysaccharides, among which chitosan, hyaluronic acid (HA), heparin, cellulose sulfate, alginic acid, dextran sulfate, and carboxymethylcellulose are most common in encapsulation of cells. Recently, cell membrane, which contains various natural polyelectrolytes, can also be utilized as the building block for LbL self-assembly.[54–56] A variety of cell membranes have been widely utilized to encapsulate different nanoparticles for enhanced nanoparticle functionalization[57] or targeted drug delivery.[58,59]

2.3.2. Nanoparticles

Nanoparticles utilized in LbL self-assembly technique for cell encapsulation are derived from stabilized colloidal dispersions of silica,[60] metal nanoparticles,[61] polyoxometalates,[62] carbon nanotubes,[63] and magnetic nanorods.[64] The process of LbL encapsulation of cells with nanoparticles is quite similar to the process of LbL encapsulation of cells with polyelectrolytes. The major difference is that certain polyelectrolyte layer is replaced with a layer of charged nanoparticles. The electrostatic attraction between the polyelectrolyte and charged nanoparticles could facilitate the adsorption of charged nanoparticles onto cell surface, support the stability of the architecture, and prevent the internalization of nanoparticle into the cytoplasm. Recently, some researchers also reported the direct deposition of polyelectrolyte-stabilized nanoparticles onto cells by electrostatic interaction to achieve cell surface functionalization.[65]

2.4. Assembly Technologies of LbL Self-Assembly Technique for Cell Encapsulation

The need to meet different processing requirements has facilitated the design and development of various LbL self-assembly technologies. Generally, we can classify various assembly technologies into five major categories, including (i) immersive,

(ii) spin, (iii) spray, (iv) electromagnetic driven, and (v) fluidic assembly.[66,67] Among these five major assembly technologies, immersive assembly and fluidic assembly are two major methods that can be applied to cell encapsulation. Immersive assembly, which is also called dip assembly, is the most widely utilized LbL assembly method for cell encapsulation. This assembly technology is performed by immersing cells into the desired material solution for a period of time, then followed by centrifugation and washing to remove the unbound material.[68,69] The simplicity of immersive assembly technology to form interpenetrated nanocoating on cell surface in mild conditions makes it easily accessible.[67] As the most widely utilized method for LbL cell encapsulation, immersive assembly will continue to play an important role in the LbL cell encapsulation. Fluidic assembly technique can perform multilayer assembly on cell surfaces by loading cells into the fluidic channels, then followed by using vacuum or pressure to drive the sequential flow of polymers and washing solutions through the fluidic channels to achieve cell encapsulation.[70–73] The microfluidic platform is made up of channels with micrometers dimensions, which provides a fast and new method for cell encapsulation and can significantly decrease the reagent consumption and waste production.[74,75] Although this technology requires special equipment and expertise, fluidic assembly method greatly improves the efficiency of LbL multilayer assembly through coating various cells with decreased reagent consumption. Compared with the immersive assembly which usually takes 10–20 min to deposit each layer and has many deposition and washing steps, microfluidic assembly could significantly reduce the total assembly time to 1–2 min by changing the solution flow rate.[71,72] Due to the constantly flowing liquid environment which enables fully contact between polymers and encapsulated cell, microfluidic assembly could achieve more homogeneous nanocoating on cells when compared with the immersive assembly.[76] Additionally, the microfluidic assembly applies a very low sheer stress, low reaction reagent volume, and generally no centrifugation step, which could minimize cell damage and maintain high cell viability.[77]

3. Strategies of LbL Self-Assembly Technique for Cell Encapsulation

Based on the difference in encapsulation strategy, we can generally divide LbL cell encapsulation into two main categories (as shown in **Figure 2**): direct cell encapsulation and indirect cell encapsulation.[68] The direct cell encapsulation technique refers to the sequential deposition of predetermined polymers to form multilayered nanofilms on single cells or cells aggregates to achieve single cell encapsulation or cell aggregate encapsulation. The indirect cell encapsulation technique involves the initial encapsulation of cells within a biocompatible hydrogel core, then followed by the LbL self-assembly of polymer on the hydrogel to form the multilayer shells. Based on the different disposal ways of the hydrogel core, the indirect cell encapsulation can be sorted into two distinct functional structures: (1) LbL encapsulated bulk hydrogel, in which the hydrogel core still exists in the final application; (2) LbL encapsulated hollow capsule, in which after the LbL encapsulation of the multilayered polymers on the hydrogel, the hydrogel core is sacrificed by hydrogel liquefaction to obtain a hollow core–shell structure.

3.1. Direct Cell Encapsulation

Direct cell encapsulation via LbL self-assembly technique involves the sequential deposition of polyelectrolytes or complementary materials on cell surface through intermolecular interactions including the electrostatic attraction between negatively charged cell membrane and oppositely charged polyelectrolytes,[48] hydrogen bonding,[78] covalent bonding,[79] etc. In general, electrostatic LbL assembly is the most widely utilized technique to encapsulate cells. Electrostatic LbL assembly usually starts with the deposition of a polycation on the negatively charged cell membrane as the first layer, then followed by sequential deposition of polyanion and polycation until the planned shell architecture and desired shell thickness being achieved. The desired thickness and architecture of shells could be controlled by modulating the types of polyelectrolytes and deposition steps. The whole encapsulation process is performed in mild conditions to achieve fast cell encapsulation with tunable structures and controllable properties to meet various biomedical applications. Due to the direct contact between the first polycationic layer and cell membrane, the cytotoxicity of polycations may be a possible issue of electrostatic LbL assembly, as direct exposure of mammalian

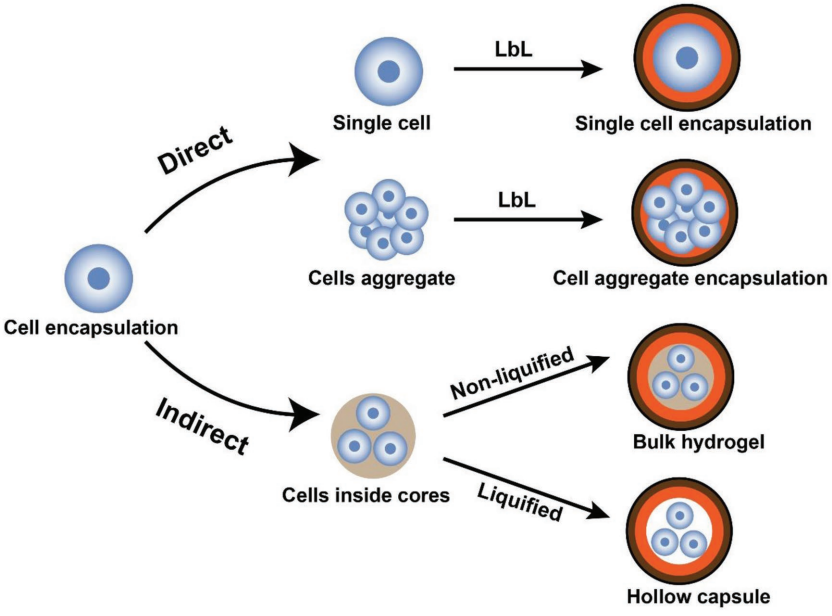

Figure 2. Schematics showing different strategies of LbL self-assembly technique for cell encapsulation. Single cells or cell aggregates can be directly encapsulated by LbL self-assembly technique, or initially encapsulated within biocompatible materials (such as hydrogel and inorganic material), then followed by LbL self-assembly on the surface of the material to form multilayer shell.

cell membrane to polycations might lead to cell membrane perforation.[68] The cytotoxicity of polycations mainly depends on the molecular weight and charge density.[80] Nevertheless, it has been demonstrated that natural cationic polyelectrolytes exhibit excellent cytocompatibility with no detectable cytotoxicity.[27] Additionally, the cytotoxicity of synthesized polycations could be significantly reduced by conjugating neutral molecules (such as poly(ethylene glycol) (PEG)) to the polyelectrolyte structure to decrease the charge density and increase the molecular weight.[48]

3.1.1. Single Cell Encapsulation

The earliest work on single cell encapsulation by LbL self-assembly technique dates back to 2002 when Lvov and co-workers reported the encapsulation of bovine platelet with PDDA and PSS.[81] They demonstrated that adjusting the thickness and components of the shell could inhibit platelet aggregation and controls platelet secretion. Such result shed light on the feasibility of manipulating the function of single cells by LbL cell encapsulation. From then on, several researches focused on the encapsulation of yeast and bacterial spore by LbL assembly and observe the viability and metabolic activity changes of the encapsulated cells.[82–85] For example, Diaspro et al. reported the encapsulation of yeast (*S. cerevisiae*) with PAH and PSS and observed that microbial cells maintained their normal metabolic activities and viability after encapsulation.[86] They also discovered that microbial cell growth and division could be controlled by tuning the properties of the polyelectrolyte shell. After achieving the LbL encapsulation of yeast and bacteria, some researches concentrated on realizing cell surface functionalization by LbL deposition of functional nanoparticles on cells and reducing the encapsulation time. Fakhrullin's group has done a great many pioneering works on the construction of magnetically functionalized cells[65,87] and developed the time-saving single-step deposition of polyelectrolyte-stabilized nanoparticles on cells.[64,88,89] For example, Konnova et al. reported the rapid one-step construction of magnet-responsive bacteria by directly depositing PAH-stabilized iron oxide nanoparticles on bacteria using the electrostatic interaction between the cell surface and PAH-stabilized nanoparticles.[65]

These works on the encapsulation of platelet,[81,90] yeast,[63,64,91–94] and bacteria[84,95,96] by LbL assembly technique demonstrated the potential of the artificial multilayer shell in cell protection, cell viability maintenance, cell proliferation and division regulation, and cell surface functionalization.[97,98] The capability to fabricate shells of any constituents in a predefined order enables the precise manipulation of the physicochemical properties of the multilayer shell and could have the intrinsic functions of these surface functionalized cells enhanced or altered.[99–101]

After the successful encapsulation of platelet, yeast, and bacteria by LbL assembly technique, research direction turned to the LbL encapsulation of various mammalian cells, including human cells. Unlike bacterial cells which have the thick and resilient cell walls, mammalian cells do not have the polysaccharide-strengthened cell wall, thus having poor resistance to the osmotic pressure and mechanical stress.[27] Therefore, it is necessary to be more careful when choosing LbL assembly materials for mammalian cells encapsulation. Nevertheless, the encapsulation of mammalian cells by LbL assembly technique was finally achieved.[102] A variety of mammalian cells, including erythrocyte,[103,104] fibroblast cell,[105] endothelial cell,[106] stem cell,[107] immune cell,[108] cancerous cell,[109,110] etc., have been used for cell encapsulation. A variety of LbL assembly materials, including synthetic polyelectrolytes and natural polyelectrolytes, nanoparticles, etc., have been utilized in cell encapsulation.[47,111] Early work on LbL encapsulation of mammalian cells was mainly achieved by electrostatic interactions between the negatively charged cell membrane and the polycationic first layer. However, the possible cytotoxicity of some synthetic polycationic polymers has stimulated the development of non-electrostatic LbL assembly methods utilizing intermolecular interactions such as covalent bonding and hydrogen bonding. As for hydrogen-bonded multilayer assembly, the first layer of polymer was deposited on the cell surface through hydrogen-bonded interaction between polymer and cell membrane proteins (such as collagen), then followed by deposition of other polymers through the hydrogen-bonded interaction between layers.[78,112,113] Previous studies have demonstrated the hydrogen-bonded LbL encapsulation of pancreatic islet cells and yeast cells through hydrogen bonding between the hydroxyl groups of tannic acid (TA) and the carbonyl groups of poly(N-vinylpyrrolidone) (PVPON).[112,113] Compared with electrostatic LbL assembly, the resulted multilayer could significantly increase the cell viability and preserve long-term cell functioning. Furthermore, covalent-bonded LbL assembly was performed by covalent bonding between polymers. Typically, the first layer of polymer was deposited on cell surface through the covalent bonding between polymer and cell surface protein (such as integrin).[68] Akashi's group has fabricated various covalent-bonded LbL cell encapsulation systems based on the interaction between fibronectin (FN) and gelatin (G), two extracellular matrix proteins.[114–116] The RGD sequence in FN could covalently bind to cell surface integrins, which resulted in the deposition of first FN layer on cell surface. FN also possesses a collagen binding domain which could covalently bind to G, thus forming multilayers of FN-G on the encapsulated cells with good cytocompatibility. The versatility of the LbL technique to tailor the components and structure of the multilayer shells allows for various applications of the encapsulated cell, including biosensor, cell therapy, drug delivery, etc.[28,48]

3.1.2. Cell Aggregate Encapsulation

Apart from single cell encapsulation, LbL assembly technique can also be applied for larger cell aggregate encapsulation, such as pancreatic islets, serving as transplants with increased resistance to the host immune response.[117] The initialization of pancreatic islets dates back to 1990s, when a clinical study first discovered that the transplantation of encapsulated islets within alginate hydrogels could significantly reduce the exogenous insulin requirement in a type-1 diabetic patient.[118] However, there exists some limitations in the bulk hydrogel based cell aggregate encapsulation strategy, including hypoxic cell death, incomplete immune protection, poor oxygen and nutrients

diffusion, large and unsuitable volume for transplantation, and insufficient biocompatibility of the coating materials.[29] Since LbL semipermeable polymer shells could offer efficient transfer of oxygen, nutrients, and not substantially increase the volume of encapsulated islet. On this account, LbL assembly technique was applied as an alternative strategy to avoid problems related to conventional pancreatic islet encapsulation methods.[119]

Early studies with LBL encapsulation of islets employed nanothin polyion complex membranes to enclose the islets.[120,121] For example, Krol et al. reported the LbL encapsulation of human pancreatic islets in a PAH/PSS/PAH multilayer shell to protect islets function and viability.[120] Another group explored the LbL encapsulation of islets with poly(ethylene glycol)–phospholipid (PEG–lipids)/alginate/PLL multilayer complexes and found that the function of islets to release insulin after glucose stimulation was not impaired by the encapsulation.[121,122] Since then, several researches focused on the utilization of polymer–PEG–lipids architecture to form biocompatible cell aggregate encapsulation.[122,123] Other researches concentrated on the in vivo biocompatibility, cell viability, and insulin release function of islet cells after LbL encapsulation.[26,124] The increasing development of the LbL encapsulation of islet cells has driven the innovation of LbL encapsulation techniques as well. To overcome the shortcomings of aqueous LbL assembly techniques (including centrifugation, microfluidics, and selective withdrawal) in islet encapsulation, an automated filtration and wash process for LbL encapsulation of pancreatic islets was developed to provide increased encapsulation efficiency, high islet viability and structural integrity, and reduced laborious processing tasks.[72]

3.2. Indirect Cell Encapsulation

Indirect cell encapsulation technique includes LbL encapsulated bulk hydrogels and LbL encapsulated hollow capsules. In terms of LbL encapsulated bulk hydrogels, LbL encapsulation on the hydrogel performs these functions: stabilizing hydrogel's structure, better control over molecules transfer, or enhancing the integration of the hydrogel in the implantation site. However, some bulk hydrogels used for cell encapsulation have the intrinsic limitation of low permeability, which might influence the oxygen and materials diffusion. Such problem has been reported in the pancreatic islet encapsulation with alginate hydrogels, where delayed insulin secretion and core hypoxia have been observed.[125] Such problem can be solved by the design of LbL encapsulated hollow capsules, where hydrogels used for cell encapsulation function as sacrificial templates for the construction of hollow core-shell structure, and are liquefied after LbL assembly. The permeability of the hollow capsule merely depends on the intrinsic characteristics of LbL multilayer shells.

3.2.1. LbL Encapsulated Bulk Hydrogel

Since LbL encapsulation on the hydrogel core could stabilize the hydrogel structure and enhance the integration of the hydrogel in the implantation site, LbL encapsulated bulk hydrogels are primarily used for pancreatic islets and microbes encapsulation. For example, coating the alginate hydrogels encapsulating yeast cells with an ultrathin but robust LbL polydopamine shell could effectively reduce the growth rate of microbial cell and control their release, thus preventing the encapsulated microbes from undesired exposure.[126] LbL encapsulation performed on hydrogels containing pancreatic islets could achieve better control over the swelling and degradation of hydrogels.[127] Future development of LbL encapsulated bulk hydrogels may focus on the fine control and tunability of these physicochemical properties, such as permeability, stickiness, robustness, and lubrication.

3.2.2. LbL Encapsulated Hollow Capsule

After the LbL deposition of multilayer nanostructured polymers on the hydrogel, the sacrificial hydrogel core can be liquefied to produce a hollow core–shell structure.[128,129] The LbL deposition of polyelectrolytes onto the hydrogel serving as a membrane scaffold could maintain the spherical shape of the capsule after dissolution of the core and improve the biocompatibility and permeability of the capsule. This strategy may be of particular importance in applications that require high permeability of molecules to prevent core cell from threats such as hypoxia, nutrients shortage, or toxic metabolites accumulation.

LbL encapsulated hollow capsules have been widely utilized in microbial and mammalian cells encapsulation. For example, living *E. coli* cells were encapsulated in calcium carbonate microspheres.[130] After LbL deposition of polyelectrolytes on the microsphere, the calcium carbonate core was removed with ethylenediaminetetraacetic acid (EDTA) to form the hollow capsule. The encapsulated cells retained viability and showed an elongation in the lag phase. Fibroblast cells which were coated in alginate microspheres were subsequently LbL deposited with alginate and chitosan polyelectrolytes.[128] Then the alginate core was liquefied to form the hollow capsule. And cells inside the liquefied hollow capsule exhibited high viability rates. The problem of encapsulating adherent cells within a liquefied hollow capsule is the unsuitability of long time cell culture in the suspension state. To solve the problem, fibroblast cells were encapsulated in a LbL chitosan/alginate multilayer hollow capsule which incorporated poly(L-lactic acid) nanoparticles to provide sites for cell adhesion.[129] This pioneering research represented the first report that anchorage-dependent cells could adhere and proliferate on the liquefied core of a LbL encapsulated hollow capsule which exhibited high permeability and immunoprotective features. Such liquefied capsule has also been used in tissue engineering, including for the processing of cartilage-like microtissues by encapsulating microparticles with stem cells inside liquefied capsules to create a closed chondromimetic environment.[131] In the future, this technique will offer new strategy for cell aggregate encapsulation of pancreatic islets, which could incorporate pancreas native proteins in the liquefied medium surrounding the islets.

4. Biomedical Applications of LbL Self-Assembly for Cell Encapsulation

The versatility of LbL self-assembly technique for cell encapsulation makes it widely used for various biomedical applications

including cell transplantation,[122] drug delivery,[132,133] tissue engineering,[69,106,134] biosensors,[135] and so on. The capacity to modulate the physicochemical and topographical properties of the multilayer shell by changing assembly parameters and solution properties, not only allows LbL self-assembly to be suitable to protect encapsulated cells from external stimuli, but also offers a new strategy for creating nanostructured architectures for individual cells or cell aggregates for tissue engineering. Here, we focus on several recently reported biomedical applications of LbL cell encapsulation in cell-based biosensor, cell transplantation, cell/molecule delivery, and tissue engineering.

4.1. Cell-Based Biosensor

Biosensor refers to a biological sensing platform which can detect certain substance in the environment and then transduce the detection results into electrical, thermal, or optical signals.[136,137] Cell-based biosensors are generally constructed by incorporating viable functional cells as biological sensing elements to detect certain chemicals, and some methods have been developed to construct cell-based biosensors.[138,139] Conventional cell immobilization methods which are generally based on cross-linking or matrix incorporation could significantly reduce cell viability and are irreversible. On the contrary, LbL-based method enables the reversible immobilization of cells on biosensor surfaces and could maintain high cell viability.[27] In recent years, LbL encapsulation of eukaryotic cells has been utilized to protect cells in biosensors against surrounding medium for applications such as genotoxicity screening, hormone detection, etc.[135,140] For example, Garcia-Alons et al. reported that yeast bioreporter cells encapsulated within PAH-stabilized magnetic nanoparticles were used to detect the genotoxic chemicals (as shown in **Figure 3**).[135] Magnetically modified yeast cells were immobilized and retained in the microfluidic chambers by the external magnetic field and then used for genotoxicity screening. The magnetically enhanced retention, facile removal and reloading properties of the LbL encapsulated yeast cells permitted convenient and rapid toxicity screening of various chemicals. Recently, Germain et al. reported the successful protection of mammalian MELN cell line which was used in a biosensor system to detect the xenoestrogens.[140] The mammalian MELN cells were sequentially deposited with PDDA and PSS to a form a multilayer shell. The LbL multilayer shell permitted high cell survival without affecting the essential metabolic function of cells. The LbL cell encapsulation technique offers a new strategy for the design of cell-based biosensors and other cell-based devices. Future work should focus on more optimal selection of polymers which could maintain long-term function and viability of encapsulated cells.

4.2. Cell Transplantation

Since LbL encapsulation of live cells within a biocompatible semipermeable shell could attenuate deleterious host immune responses toward encapsulated cells, LbL assembly has emerged as an ideal technique for cell transplantation. Compared with conventional microencapsulation methods which usually increased the total transplant volume to several hundreds of milliliters, LbL assembly could greatly minimize the transplant volume for cell transplantation.[141] The most commonly transplanted cells using LbL encapsulation are the pancreatic islets for diabetes mellitus treatment.[26,29,120,122,142,143] For example, Wilson et al. reported the successful intraportal islet transplantation by LbL self-assembly of PLL-*g*-PEG (biotin) and streptavidin (as shown in **Figure 4**A,B).[119] The resulted nanothin coatings can be tuned to encapsulate islets without loss of viability and function.

Apart from islet encapsulation, LbL assembly can also be used to encapsulate red blood cells to attenuate immune response during blood transfusion.[103,104] For example, Mansouri et al. reported the LbL assembly of nonimmunogenic polyelectrolyte multilayer shells to encapsulate fully functional human red blood cells (as shown in Figure 4C,D).[104] Red blood cell agglutination was inhibited by the resulted nonimmunogenic shell, and the physicochemical properties of cells, such as osmotic fragility, deformability, and hydrophobicity, were not changed by shell. This method not only created an advanced nanomaterial for production of universal red blood cells, but also provided a new strategy for the design of functional multilayers for cell encapsulation.

4.3. Cell/Molecule Delivery

Since the LbL assembly multilayer shells could protect encapsulated cells from harsh environmental stimuli, LbL encapsulation

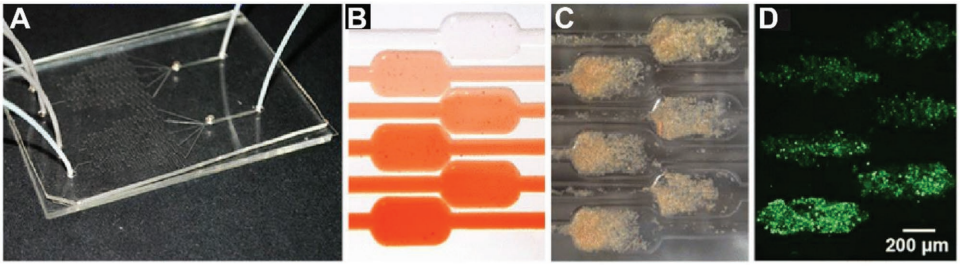

Figure 3. Microscreening toxicity system based on LbL encapsulation of yeast bioreporter cells within magnetic nanoparticles. A) Photograph of the structure of the microscreening toxicity system; B) Visualization of the generated concentration gradients within microchambers with red ink at the working flow rate; C) LbL encapsulated magnetic yeast cells were loaded into and retained microchambers for genotoxicity screening by the external magnetic field; D) Fluorescence emission from green fluorescent protein reporter yeasts. The fluorescent intensity correlated with the amount of genotoxic compounds. Adapted with permission.[135] Copyright 2011, Springer.

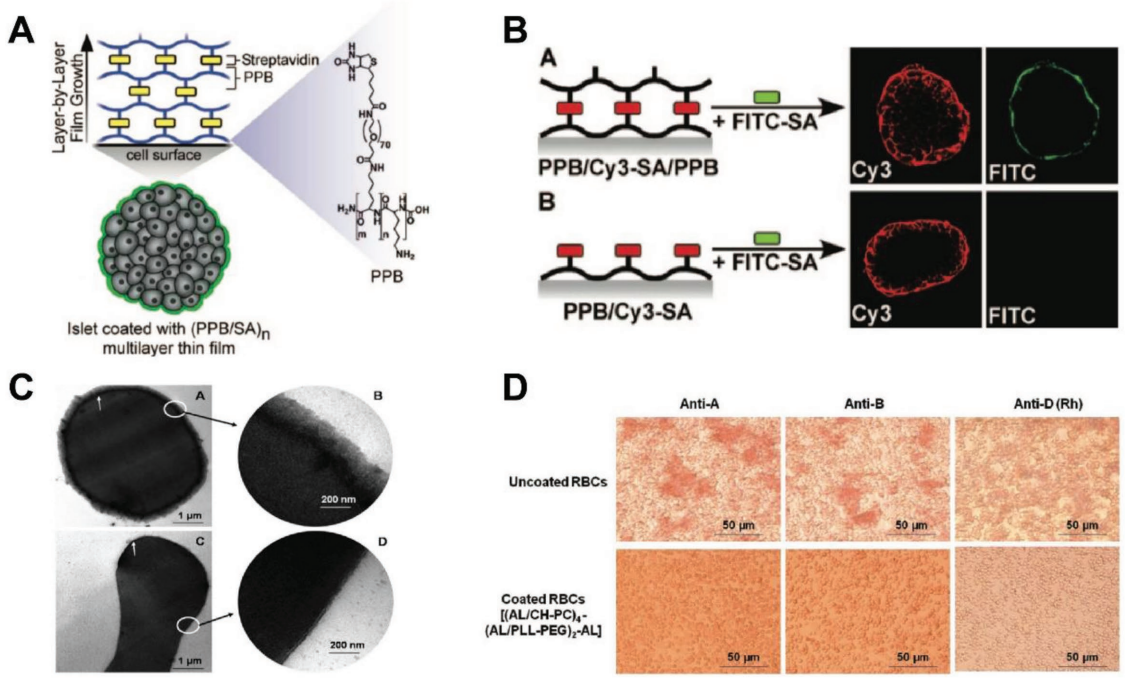

Figure 4. A) Schematic illustration of the preparation of PEG-rich, nanothin conformal islet multilayer film by LbL self-assembly; B) Demonstration of individual pancreatic islets encapsulated within PLL–PEG(biotin)–streptavidin multilayer films. Adapted with permission.[119] Copyright 2008, American Chemical Society. C) Transmission electron microscope (TEM) images of red blood cells coated with LbL assembly films (upper part) and uncoated red blood cells (lower part); D) Agglutination assay result of uncoated and coated red blood cells. Agglutination was inhibited by LbL cell encapsulation. Adapted with permission.[104] Copyright 2011, American Chemical Society.

of probiotic microbes can be used for probiotics delivery to the gastrointestinal tract. Recent years have witnessed progress in discovering the importance of probiotics in regulating human health and disease.[144] However, due to acidic stomach condition and bile salts, probiotics would quickly lose cell viability during gastrointestinal transit, thus limiting the clinical applications of probiotics.[145] To solve this problem, recent efforts have been made on encapsulating probiotic microbes by LbL techniques.[132,146,147] For example, Aaron et al. recently reported the LbL encapsulation of individual probiotic microbes to protect them in the gastrointestinal tract and promote microbial adhesion and growth in the targeted sites (as shown in **Figure 5**A–C).[132] The probiotic strain *Bacillus coagulans* was sequentially encapsulated with chitosan and alginate to form a multilayer shell. The in vivo animal experiment demonstrated the improved survival and delivery of probiotics in the gastrointestinal tract. This method offered a new choice for probiotics delivery to the gastrointestinal microbiome to improve human health.

Apart from probiotics delivery, LbL self-assembly technique can also be utilized to encapsulate therapeutic cells with functional molecules incorporated multilayer shells to achieve simultaneous delivery of cells and regulatory factors.[148–150] For example, our group previously reported the incorporation of insulin-like growth factor-1 (IGF-1) into a gelatin/alginate multilayer shell which encapsulated the neural stem cells (NSCs),[148] as shown in Figure 5D. IGF-1 was a regulatory molecule which could maintain the viability and enhance the proliferation of NSCs. We demonstrated that IGF-1 loaded into the shell could be released into the medium in a PH- and time-dependent manner, thus significantly enhancing the survival and proliferation of the encapsulated NSCs. Such model demonstrated the potential of LbL encapsulation of NSCs with regulatory molecules to treat nervous system diseases. Similarly, Liu et al. recently prepared a vascular endothelial growth factor (VEGF) delivery system targeting the myocardial infarction sites by incorporating VEGF into a gelatin/alginate LbL assembly shell which encapsulated mesenchymal stem cells (MSCs),[150] as shown in Figure 5E. MSCs had an inherent tropism for the myocardial infarction zone, and VEGF was a regulatory factor which could enhance cardiac function and promoting angiogenesis. The VEGF-encapsulated MSCs system was demonstrated to have a sustained release of VEGF and targeting to the myocardial infarction sites, thus promoting angiogenesis and enhancing cardiac function. This study provided a promising strategy for treating myocardial infarction.

4.4. Tissue Engineering

Due to the versatility and tunability of polymer materials for LbL self-assembly, LbL encapsulation of cells with extracellular matrix components has been used to mimic the structure of living tissue for tissue engineering.[116,151–154] Biocompatible extracellular matrix components such as fibronectin and gelatin have been widely used as building blocks for LbL cell encapsulation. Fibronectin and gelatin were used to form a biomimetic extracellular matrix layer on cell surfaces, providing a cell attachment platform for other types of cells to form the biomimetic

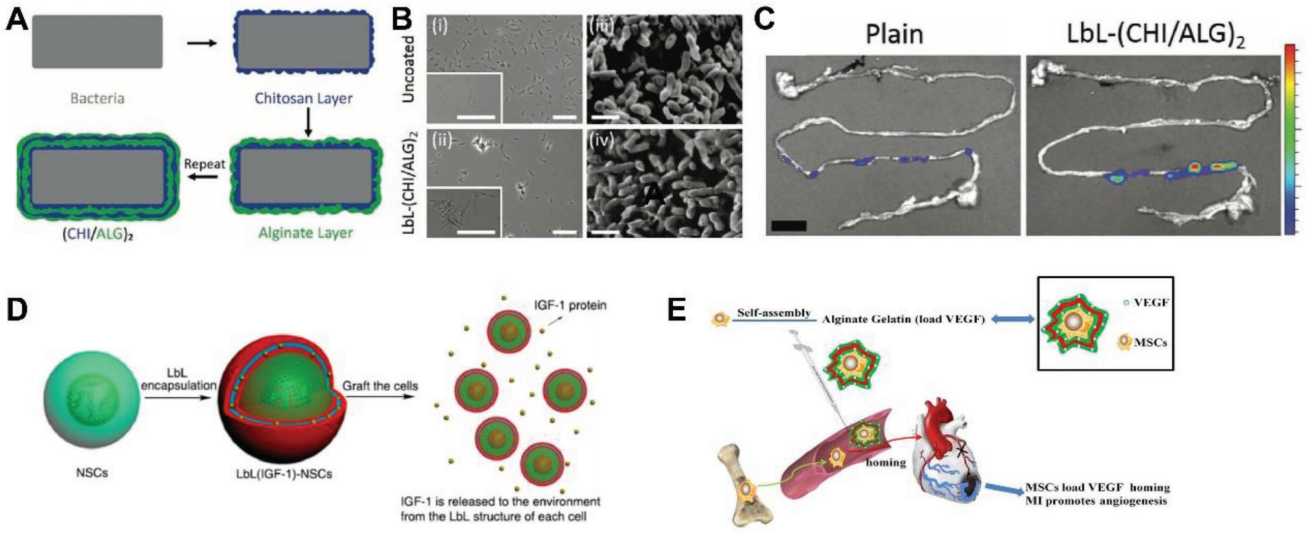

Figure 5. A) Schematic illustration of LbL encapsulation of probiotic microbial cells. B) Bright-field and scanning electron microscope (SEM) images of uncoated and coated probiotics; C) Representative in vivo imaging system images of plain and coated probiotics at 1 h after oral gavage, indicating the enhanced survival of coated probiotics in vivo. Adapted with permission.[132] Copyright 2016, Wiley-VCH. D) Schematic illustration of the design and fabrication of IGF-1 loaded LbL assembly multilayer shell which encapsulated NSCs. Adapted with permission.[148] Copyright 2015, American Chemical Society. E) Schematic illustration of the design and fabrication of a VEGF delivery system based on the LbL encapsulation of MSCs to target myocardial infarction sites. Adapted with permission.[150] Copyright 2017, Elsevier Ltd.

tissue.[106,114,155] For example, Matsusaki et al. reported the fabrication of well-organized and self-standing cellular multilayers by creating nanometer-sized fibronectin–gelatin films on the surface of each cell layer using LbL assembly,[155] as shown in **Figure 6**A. Xenogeneic cellular multilayers architectures such as blood vessels were prepared by this method. In a novel technique called "cell-accumulation technique" (as shown in Figure 6B), Nishiguchi et al. reported the rapid construction of 3D multilayer tissue with endothelial tube networks by encapsulating different cells with LbL coating of fibronectin–gelatin nanofilms.[106] Such simple and rapid technique will be promising for the construction of biomimetic tissues for tissue engineering.

Apart from constructing biomimetic tissues by LbL encapsulating cell with nanometer-sized fibronectin–gelatin films, biomimetic tissues can also be prepared by physical cross-linking on LbL encapsulated cells to form the cell spheroids.[156,157] For example, our group previously reported the fabrication of dermal papilla spheroids for hair follicle regeneration,[157] as shown in Figure 6C. Individual dermal papilla cells were encapsulated with gelatin and alginate by LbL assembly to form ultrathin nanocoated matrices. After exposure to Ca^{2+}, the outmost alginate layer would be ionically cross linked, which induced LbL encapsulated single cell to aggregate into cell spheroids. The resulted dermal papilla spheroids were morphologically and physiologically similar to primary dermal papilla. Furthermore, dermal papilla spheroids exhibited an excellent hair follicle inductive capability in vivo. This model not only provided a feasible strategy for the preparation of 3D microtissue mimics by LbL encapsulation of cells but also showed potential applications for tissue engineering.

Furthermore, biomimetic tissues can also be prepared by the layer-by-layer paper-stacking strategy.[158–162] The LbL paper-stacking strategy refers to the LbL deposition of nanomembrane onto cells to fabricate 3D biomimetic tissues or scaffolds. For example, our group previously reported the construction of biomimetic tissues by the assembly of LbL paper-stacking ultrathin extracellular matrix on a freestanding cell sheet.[158] The resulted biomimetic tissue was well organized, and could be easily peeled from the substrate upon temperature change. Besides, the LbL paper-stacking nanomembrane could efficiently support the cell sheet and maintain cell viability and cell proliferation. Furthermore, the capability of the LbL paper-stacking nanomembrane to control the release of loading drugs offered a novel method to control the cell differentiation in the biomimetic tissues. Since LbL paper-stacking nanofilms were highly amenable to surface modification to match various ends in specific tissue and could control the release of drugs to affect cell fate by encapsulating cargos into the nanofilm, such strategy would no doubt provide a versatile way to fabricate biomimetic tissues for biomedical applications.

5. Conclusions and Perspectives

LbL self-assembly technique, as a simple, robust, highly efficient and versatile method, has been extensively utilized for the design and fabrication of various nanostructured films, coatings and scaffolds with tunable architectures and tailored physicochemical properties. In this review, we attempted to summarize the recent advances in the design, fabrication, and biomedical applications of LbL self-assembly technique for cell encapsulation. LbL encapsulation of individual cells or cell aggregates allows for the attenuated host immune response in cell transplantation, cytoprotection of living microorganisms such as bacteria and yeasts, incorporation of regulatory molecules to regulate cell biological behavior, and modification of cells to endow them with new structural and functional

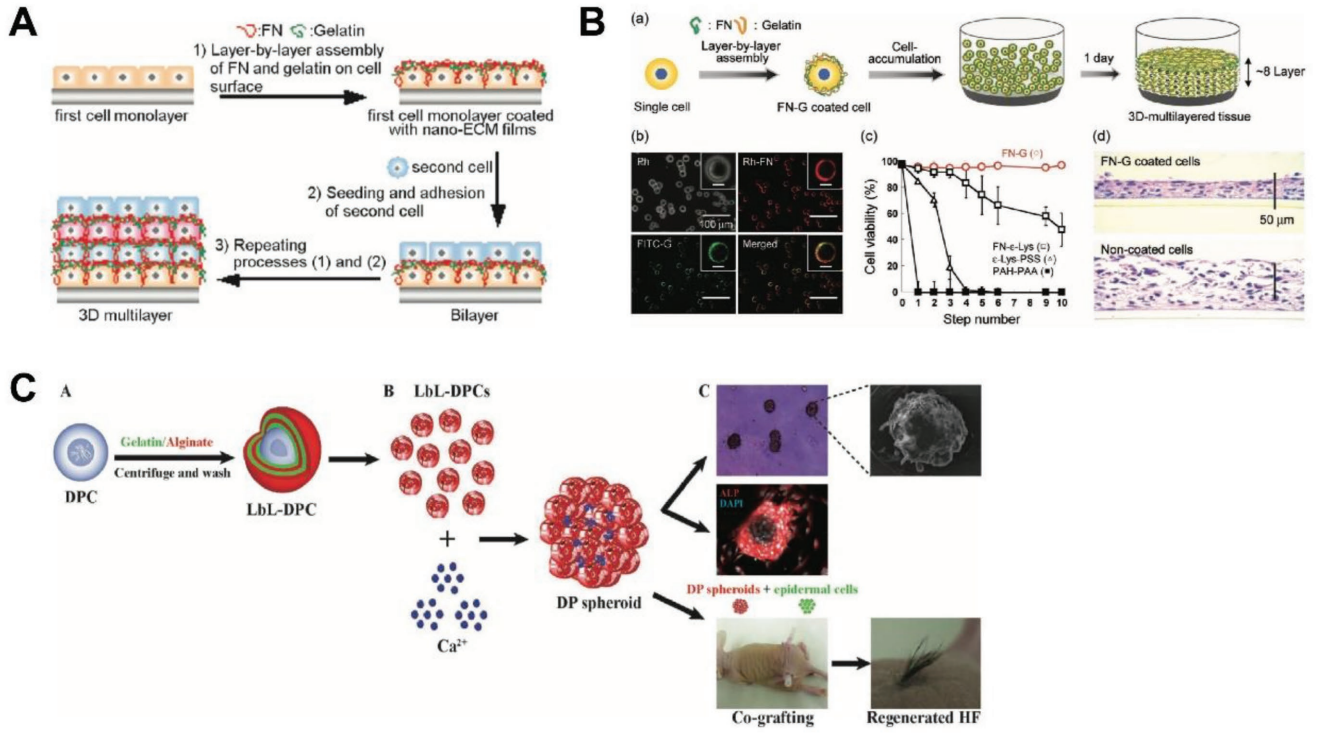

Figure 6. A) Schematic illustration of the fabrication process of 3D cellular multilayers by LbL assembly of fibronectin and gelatin on cell surface. Adapted with permission.[155] Copyright 2007, Wiley-VCH. B) Rapid construction of 3D multilayered tissues with endothelial tube networks by the cell-accumulation technique via LbL encapsulation of a single cell with fibronectin/gelatin, and the phase/fluorescent microscopic images, cell viability, and hematoxylin and eosin (H&E) staining images of the obtained tissues. Adapted with permission.[106] Copyright 2011, Wiley-VCH. C) Schematic illustration of the construction of dermal papilla spheroids by aggregation of LbL encapsulated dermal papilla cells for hair follicle regeneration. Adapted with permission.[157] Copyright 2017, Wiley-VCH.

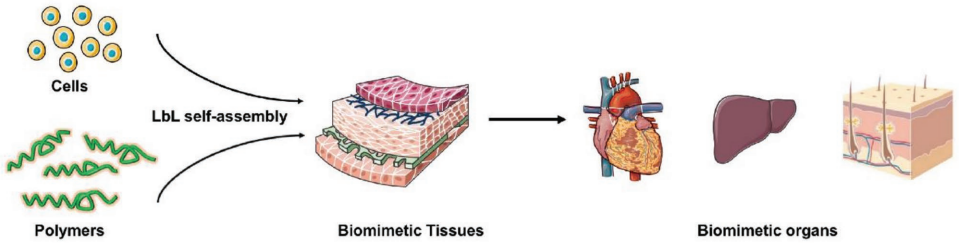

Figure 7. Future potential biomedical applications of LbL self-assembly technique for biomimetic organs construction.

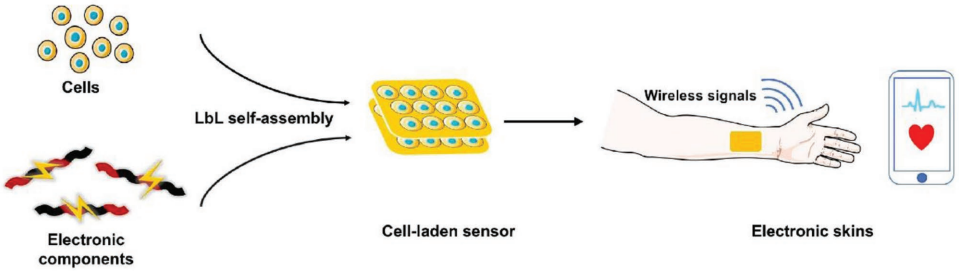

Figure 8. Future potential biomedical applications of LbL self-assembly technique for the fabrication of biomimetic electronic skins.

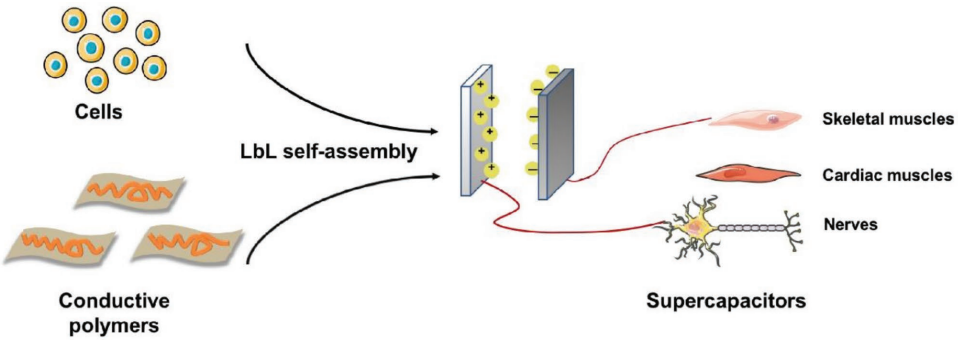

Figure 9. Future potential biomedical applications of LbL self-assembly technique for the construction of supercapacitors which can store and release bioelectricity to stimulate skeletal muscles, cardiac muscles, and nerves when needed.

features to construct desired architectures. Due to the versatility and tunability of multilayer shells, LbL cell encapsulation has been developed for various biomedical applications, such as cell-based biosensor, cell transplantation, cell/molecule delivery, and tissue engineering.

5.1. Perspectives on the Future Development of LbL Cell Encapsulation Technique

Although remarkable progress has been made in the development of LbL cell encapsulation, undoubtedly, there still exists some challenges which might restrict us to taking full advantage of the LbL cell encapsulation for constructing tailored multilayer systems with superior performances for practical biomedical applications. Remaining challenges include the optimization of process parameters for LbL cell encapsulation to obtain faster and more stable coating to permit long-term cell viability of multilayer systems. Furthermore, another challenge regarding the LbL cell encapsulation technique is the design of highly flexible multilayer shells that allow shells to still entirely encapsulate cells even after cell division. Moreover, new strategies and coating materials for LbL cell encapsulation should be pursued to obtain multilayer system with novel structures and physiochemical properties to meet the requirements of new applications. For example, due to the biocompatible, targeting, and immune camouflaged features, cell membranes have been utilized as the building blocks to encapsulate different nanoparticles.[56,59] In the future, we could utilize cell membranes for the LbL encapsulation of various cells to construct biomimetic tissues. In addition, the integration of LbL self-assembly technique and other multidisciplinary fabrication techniques should be explored to develop new strategies for cell encapsulation. For example, LbL self-assembly technique can collaborate with artificial intelligence (AI) technology or 3D bioprinting technique to achieve intelligent, automated, massive, and highly efficient fabrication of multifunctional smart biomimetic materials for a variety of biomedical applications. Besides, LbL self-assembly technique can also be utilized to fabricate novel biomimetic stimuli-responsive smart materials with controllable structures and tunable functions in the near future.

5.2. Perspectives on the Future Biomedical Applications of LbL Cell Encapsulation Technique

Apart from concerns on the future development of LbL cell encapsulation techniques, we also envisage several future potential biomedical applications of LbL cell encapsulation technique as specifically elucidated in **Table 1**.

First of all, thus far, the vast majority of the reported researches are mainly concerned with the construction of biomimetic tissues by LbL encapsulation of cells within multilayer functional shells. However, compared with the native tissues, the structure and function of the biomimetic tissues constructed by the LbL cell encapsulation technique are still far from satisfactory. Besides, there is currently no report on the successful fabrication of biomimetic organs by the LbL cell encapsulation technique. In this regard, it is necessary for us to focus on the construction of biomimetic tissues and organs (such as skin, heart, liver) with structures and functions similar to native tissue and organ by sequentially LbL encapsulation of different layers of diverse cells and extracellular

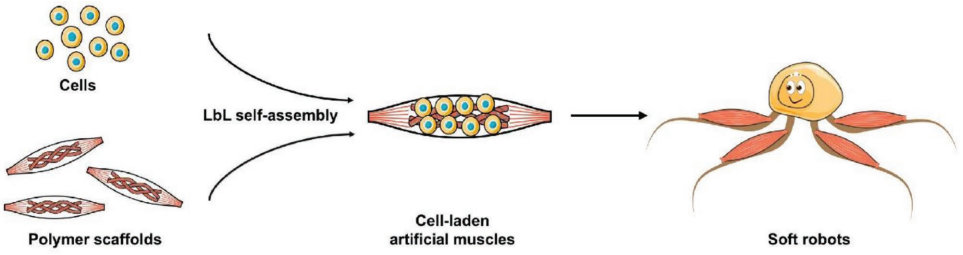

Figure 10. Future potential biomedical applications of LbL self-assembly technique for the construction of biomimetic soft robots.

Table 1. Representative examples of LbL cell encapsulation for biomedical applications.

Application	Shell	Cell	Objective	Reference
Cell-based biosensor	PDDA/PSS	MELN cell line	Protection of mammalian cell used in biosensors	[140]
	PAH-stabilized magnetic nanoparticles	Yeast cells	Magnetic immobilization and retention of yeast cells in the biosensor device	[135]
Cell transplantation	PLL-*graft*-PEG/alginate	Murine pancreatic islets	Cytocompatible encapsulation of islet cells for transplantation	[26]
	PLL-*g*-PEG(biotin)/streptavidin	Murine pancreatic islets	Encapsulation of pancreatic islets for intraportal transplantation	[119]
	PAH/PSS/PAH	Human pancreatic islets	Immune protection of human pancreatic islets	[120]
	Poly(vinyl alcohol)/PEG–lipids	Syrian hamsters pancreatic islets	Encapsulation of pancreatic islets for transplantation	[122]
	Biotin–PEG–N-hydroxysuccinimide (NHS)/streptavidin/biotin–PEG–peptide	Murine pancreatic islets	Encapsulation of pancreatic islets for transplantation	[143]
	Chitosan-*graft*-phosphorylcholine/PLL–PEG/alginate	Red blood cell	Immunocamouflage of blood group antigens	[104]
Cell/molecule delivery	Chitosan/alginate	*Bacillus coagulans*	Protection of microbes for probiotic delivery	[132]
	Chitosan/sulfated β-glucan	*Lactobacillus acidophilus*	Protection of microbes for probiotic delivery	[146]
	Chitosan/carboxymethyl cellulose	*Lactobacillus acidophilus*	Protection of microbes for probiotic delivery	[147]
	Gelatin/alginate	Rat neural stem cells (NSC)	IGF-1 loaded to the LbL coating to enhance NSC proliferation	[148]
	Gelatin/alginate	Mouse dermal papilla cells (DPC)	FGF-2 loaded to the LbL coating to regulate DPC function	[149]
	Gelatin/alginate	Mesenchymal stem cells (MSC)	VEGF loaded to the LbL coating to enhance cardiac function and promoting angiogenesis	[150]
Tissue engineering	Fibronectin–gelatin	Human dermal fibroblast cells and endothelial cells	Construction of 3D vascularized tissues and human skin equivalents	[106,116]
	Fibronectin–gelatin	Insulin-secreting MIN6 cells	Fabrication of pancreatic β-cell spheroids	[151]
	Fibronectin–gelatin	Human primary hepatocytes, endothelial cells and dermal fibroblast cells	Construction of 3D vascularized human liver tissue	[114]
	Fibronectin–gelatin	Mouse L929 cells and endothelial cells	Fabrication of 3D cellular multilayers	[155]
	Gelatin/alginate	Human breast cancer cell	Construction of 3D tumor spheroids to study epithelial-to-mesenchymal transitions	[156]
	Gelatin/alginate	Mouse dermal papilla cells	Construction of dermal papilla spheroids for hair follicle regeneration	[157]
	Gelatin/chitosan/alginate	Human myoblast cells	Construction of biomimetic tissues by LbL paper-stacking strategy	[158]
	Gelatin/polycaprolactone	Mouse adipose-derived stem cell	Construction of LbL paper-stacking biomimetic tissues for bone regeneration	[159,160]
	PLL-coated graphene oxide	3T3 fibroblasts	Preparation of 3D multilayer tissue constructs by LbL paper-stacking strategy	[161]

matrixes in a specific order. It is envisaged that the construction of biomimetic organs by the sequential LbL encapsulation of diverse cells and extracellular matrixes could provide new alternative ways for the precise control over the structure of the organs and promote the further development of tissue engineering and regenerative medicine (as shown in **Figure 7**).

Second, LbL self-assembly technique can be utilized to fabricate biomimetic artificial electronic skin by LbL assembly of sensor elements with skin cells. For those people suffering from skin damage or amputations, it is of great importance to restore the sensational properties of the skin to improve the quality of their lives.[163] Such challenge has motivated the development of various novel electronic skin devices for various clinic applications, such as detecting blood pressure, body temperature, heartbeat, electrolyte balance, breathing rate, etc.[164,165] Currently, there have been several reports on

the construction of smart electronic skins akin to biological tissues.[166,167] The combination of electronics with LbL multilayers is intriguing. But we could not neglect the fact that the assembly process is performed in wet conditions and the final materials are usually highly hydrophilic, thus preventing the connection with electronic circuits and component. Fortunately, there are already some reports on the fabrication of polymer solar cells and wire-shaped supercapacitors by LbL assembly in solutions.[168,169] In the future, we envisage that the biomimetic electronic skins could be constructed by LbL assembly of sensor elements with skin cells and used as skin implants or skin accessories to real-time monitor and detect various physicochemical signals in the human body and report various diseases such as myocardial infarction, cerebral infarction, etc. Such smart device would greatly improve the health of all human beings in the future (as shown in **Figure 8**).

Third, in the future, LbL self-assembly technique can also be utilized for the construction of biocompatible micro-supercapacitors which can store and release bioelectricity to stimulate electrophysiological activity of skeletal muscles, cardiac muscles, and nerves when needed (as shown in **Figure 9**). Such micro-supercapacitor devices could function as a switch to regulate the electrophysiological activity and function of cell and have broad application prospects in the field of biomedicine. Currently, there are already reports on the fabrication of cardiac patch with microelectronics embedded to provide electric stimulus to myocardial cells.[170] In the future, for example, the combination of micro-supercapacitors with cardiomyocytes by LbL self-assembly technique could be used as cardiac pacemaker to treat heart diseases such as arrhythmia. The combination of micro-supercapacitors with nerve cells by LbL self-assembly technique could be used to treat central nervous system diseases such as epilepsy. Such strategy would no doubt facilitate the fabrication of a variety of novel nanoarchitectures for extensive practical biomedical applications.

Last but not least, note that biomimetic soft robots in recent years have sparkled increasing interest due to the high flexibility and deformability properties and have been widely used in many biomedical applications. Currently, there are some reports on the construction of soft robots which utilize sperms or myocardial cells as the nanomotors to provide the driving force.[171–173] In the near future, we envisage that LbL self-assembly technique can be utilized to fabricate biomimetic soft robots with controllable structures and tunable biomedical functions (as shown in **Figure 10**). For example, LbL self-assembly technique can be utilized to fabricate the artificial muscles which can then be attached to the soft robots to serve as the electronic muscles to adjust and control the movement of the biomimetic soft robots. Besides, LbL self-assembly technique can also be applied to encapsulate myocardial cells or skeletal muscle cells, serving as the bioactuators to power the propulsion of biomimetic soft robots. Introducing LbL self-assembly technique to the preparation of biomimetic soft robots will significantly enhance their flexibility and deformability properties to meet various biomedical application in the future.

In summary, despite remarkable achievements have been made to date in the field of LbL cell encapsulation, there are still many challenges and opportunities remaining for the further development of next generation's multifunctional biomimetic smart biomaterials with superior performance for practical biomedical applications. With the joint efforts of various scientific research fields, we envision that these challenges will be overcome. LbL self-assembly technique will be more versatile and tailored, and would significantly facilitate the development of biomedical field in the future. We hope that this review could appeal to a broad audience and motivate more scientific researchers to focus on exploring the potentialities of the LbL self-assembly technique in the biomedical field.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

cell encapsulation, layer-by-layer, nanocoating, polyelectrolytes, self-assembly

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