Expert Opinion

Layer-by-layer assembly for drug delivery and related applications

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Introduction: High-performance drug delivery systems are always made through assembly and hybridization of multiple components, each of which possesses its own role within the unified delivery function. The layer-by-layer (LbL) adsorption technique offers huge freedom in material selection and flexibility of structural design, which are fully matched with the fabrication needs of drug delivery materials requiring complicated designs.

Areas covered: In this review, film-type LbL assemblies and their drug delivery application are focused on, with selected examples from recent years. In addition to a description of the general progress of this technique in bio-related areas, examples of the delivery of low-molecular-mass drugs, DNA, peptides and proteins are summarized, as well as recent advances in film structures composed of organic/inorganic hybrids.

Expert opinion: The authors expect that the highly versatile nature of the LbL assembly can overcome any remaining practical difficulties in delivering therapeutics, because the layer structure, component selection, and the surface nature including biocompatibility, degradability and size/dimension are all adjustable. Furthermore, the simple and inexpensive nature of this technique can also satisfy strict demands from an economic point of view.

Keywords: controlled release, drug delivery, film, hybrid, layer-by-layer assembly

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1. Introduction

In many cases of conventional material release, one must think only about a couple of key factors, such as gradient of chemical potential and structural stability. In drug delivery situations, however, one must consider many more factors, including toxicity, biocompatibility, degradability and sustainability of release [1-3], and therefore the designs of such systems are typically much more complicated than those for non-biological material release. As a result, construction of drug delivery systems from a simply structured material is rather rare. Indeed, drug delivery systems with high performance are typically made through assembly and hybridization of multiple components, each of which possesses its own role within the unified function of delivery.

Bio-friendly soft materials appropriate for drug delivery are usually fabricated by self-assembly and related processes [4-9]. In many cases, biomembrane-like thin – or, more often, *ultrathin* – films in flat, curved and shell structures are used as self-assembled materials for drug delivery. Various methodologies for ultrathin-film preparation have been widely used, such as the Langmuir–Blodgett (LB) technique [10-13], the self-assembled monolayer (SAM) method [14,15] and the layer-by-layer (LbL) adsorption technique [16-18]. If one looks specifically to the use in drug delivery applications, the number of research examples based on the LbL technique is much larger than for the other two formation techniques. These is a logical consequence of the freedom in material selection and flexibility

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in structural design offered by the LbL approach, as these characteristics are well suited to the demands on fabrication of drug delivery materials that require rather complicated design in their components and structures. In addition, the apparatuses required for the LbL procedure are relatively simple and inexpensive, which further supports the viability of the approach for practical application by enabling rapid prototyping and scale-up for commercialization.

To understand this technique and its potential, it is worthwhile considering the history of developments that have led to the current state of the art. Some time after the suggestive report by Iler [19], the LbL assembly was first experimentally realized and established by Decher and Hong [20,21]. Several years later, a powerful strategy for the application of LbL to coating colloids and subsequent shell formation was invented [22-24]. As exemplified in the LbL assemblies between cationic and anionic polyelectrolytes (**Figure 1A**), adsorption of the cationic polyelectrolyte at the negatively charged surface of a solid support usually causes over-adsorption, resulting in surface charge reversal under appropriate conditions. This yields a self-limiting and highly repeatable process. Subsequent adsorption of anionic polyelectrolyte again reverses the surface charge so that alternation of the surface charge permits continuous fabrication of the layered structure. This process can also be applied to assemblies between cationic polyelectrolyte and anionic particles such as protein molecules (**Figure 1B**). It is noteworthy, however, that the driving force of self-assembly is not limited to electrostatic interaction. Various interactions including hydrogen bonding [25] and biospecific interaction [26] can be used, which expands further the options for materials and deposition conditions for film construction and also yields different and useful film properties. In addition, there is a vast choice of available materials, including biological substances such as proteins [27,28], nucleic acids [29], saccharides [30] and virus particles [31] as well as various organic polymers [32], molecular assemblies [33] and inorganic substances [34-37] for the LbL assembly. As one can imagine from the simple procedure of the LbL assembly, the assembling procedure can be performed in mild aqueous medium and does not require chemically harsh conditions. In addition, the LbL structures are less densely packed than those of LB films [38,39], which is advantageous for diffusion-drive release of drugs through the films [40]. As exemplified in nanofilm reactors [41,42] with enzymatic LbL structures, biomolecules softly fixed in the LbL films are usually more stabilized against external disturbances and show increased duration of activity.

With these characteristics of the LbL technique established, LbL assemblies have recently undergone extensive research for drug delivery applications [43,44]. Capsule-type LbL assemblies have been of particular interest, because of their potential for targeted delivery in the body. By contrast, film-type LbL assemblies cannot generally travel through the body, but they can be a very powerful methodology for coating of biomedical apparatuses that need sustained release of drugs (e.g., implanted devices such as stents). In this review, film-type LbL assemblies and their drug delivery applications are focused on, with selected examples from recent years.

2. Layer-by-layer films for biological applications

Before a description of specific drug delivery applications, recent research examples of LbL film fabrication that have prospective value for biomedical purposes are briefly summarized. Owing to the breadth of areas covered by these examples, the research outcomes and the potential implications are described without attempting to categorize them. Khutoryanskaya and co-workers prepared hydrogel-type LbL assemblies that could be used as model substrates to investigate the adhesive properties of pharmaceutical tablets. They silanized glass slides and subsequently performed LbL assembly of interpolymer complexes formed by poly(acrylic acid) and methylcellulose [45]. Thermal treatment of the films promoted crosslinking of polymers, resulting in non-detachable ultrathin hydrogels that showed pH-dependent swelling properties. Specifically, they observed minimal water uptake at pH < 6, compared with a dramatic volume increase at higher pHs and the usual dependence on sample thickness. Carlmark and co-workers synthesized thermoresponsive block polymers from *N*-isopropylacrylamide and (3-acrylamidopropyl)trimethylammonium chloride and assembled the synthetic polymers with nanofibrillated cellulose in layer-by-layer fashion [46]. Cellulose is a renewable, fibrillar nanomaterial with interesting strength and biocompatibility properties, and the composite films obtained have potential for coatings in bio-related applications, including sensors, filters, membranes and drug delivery systems. Bulwan *et al.* reported the preparation of stable single-component multilayered films of chitosan by means of the LbL technique, using two water-soluble chitosan derivatives: polycationic and polyanionic forms [47]. As chitosan derivatives have been reported to possess excellent biocompatibility and bacteriostatic properties, these LbL films could be useful for many kinds of biomedical and environmental protection applications. Kim *et al.* synthesized star polymers with oppositely charged arm structures, poly[2-(dimethylamino)ethylmethacrylate] star polymer and poly(acrylic acid) star polymer, with crosslinked cores [48]. These polymers were assembled by the LbL assembly to form non-uniform and porous structures owing to their architecture and high molecular mass as compared with conventional linear polyelectrolytes. The prepared films showed extensive structural reorganization on post-treatment with different pH conditions owing to the highly pH-sensitive nature of star polymers. The interesting architectures and high degree of functionalities of these star polymers would lead to potential applications such as vehicles for drug delivery.

Buck and Lynn used an approach of the LbL assembly for the preparation of semipermeable membranes (**Figure 2**) [49].

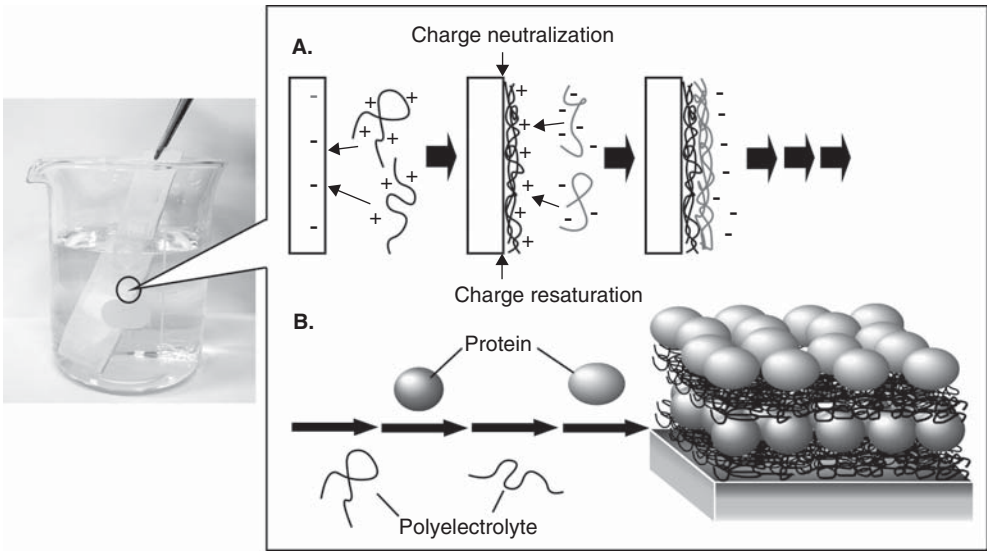

Figure 1. An example of a layer-by-layer film from (A) polyelectrolytes and (B) polyelectrolyte/protein.

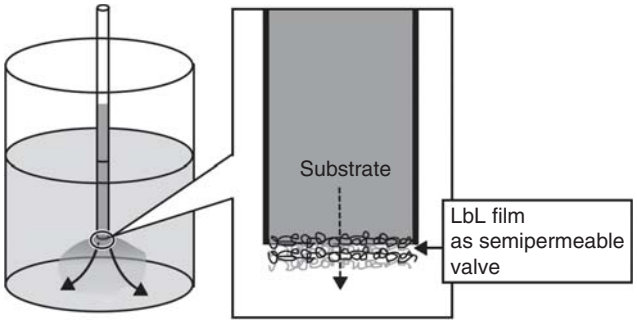

Figure 2. A layer-by-layer film controlling materials release.
LbL: Layer-by-layer.

It is based on the rapid reaction of azlactone-functionalized polymers with polymers containing primary amine functionality. Freely suspended and mechanically robust semipermeable membranes that span across the open ends of pores and orifices with dimensions on the order of tens of micrometers to several millimeters can be obtained. This approach is especially suited to the fabrication of suspended membranes within the channels of microfluidic devices or the open ends of micropipettes, which is expected to find use in biomedical separation, sensing and drug delivery. Tsai *et al.* used a sodium cholate suspension-dialysis method to adsorb the redox enzyme glucose oxidase onto single-walled carbon nanotubes and assembled them into LbL films as amperometric biosensors with a poly[(vinylpyridine)Os(bipyridyl)$_2$Cl$^{2+/3+}$] redox polymer [50]. Incorporation of single-walled carbon nanotubes increased current by twofold as compared with the response of films without carbon nanotubes. The reported improved conductivity of the composite LbL films could be useful for miniaturization of biosensors with low detection limits and for increased power output in biofuel cell applications.

As a DNA-related example, Cai and co-workers prepared gene-functionalized LbL films composed of chitosan and plasmid DNA on a titanium surface that were used to investigate the surface-mediated *in situ* differentiation of mesenchymal stem cells [51]. Functional genes were released in a sustained manner during the degradation of the LbL structures. The released genes positively stimulated the adhered mesenchymal stem cells for 2 weeks, as reflected by gene expression and protein production. This type of system has great potential in applications such as the development of gene-stimulating biomaterials and implant technology.

3. Layer-by-layer films for delivery of small drugs

There are various kinds of low-molecular-mass drug necessary for biomedical applications. Controlled release of these small drugs is a particularly important goal in the corresponding research area. For example, surgical sutures capable of drug loading and sustained release are important for wound-healing applications. Sun and co-workers proposed a facile way to incorporate ibuprofen in surgical sutures by the LbL deposition of chemically crosslinked poly(allylamine hydrochloride) and dextran with hyaluronic acid [52]. It was shown that ibuprofen incorporated in the surgical sutures can be released in normal saline in a sustainable way. In principle, the amount of drugs loaded and the release kinetics of the drugs can be adjusted by tailoring the parameters for the LbL films, though this was not demonstrated experimentally. It is noteworthy that this surface modification method does not alter the mechanical properties of the surgical suture.

Hu and Ji developed LbL films of sulfonated hyperbranched polyether with chitosan as nanoreservoirs for hydrophobic guest molecules [53]; the hydrophobic core of the polyether contributed to the affinity for guest molecule uptake. Their research found that a post-diffusion process was more efficient at incorporating hydrophobic guest molecules into the LbL films compared with the pre-encapsulation approach. A coating with potential anticoagulation, antibacterial and local release of the hydrophobic drug Probucal was investigated. Probucal is known to have powerful antioxidant properties and can prevent restenosis after coronary angioplasty. This strategy could lead to a multifunctional coating capable of anticoagulation and antibacterial and local drug delivery.

Dubas and co-workers investigated loading of curcumin into polyelectrolyte multilayer films [54]. Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione] is a yellow pigment obtained from powdered rhizomes of *Curcuma longa Linn*, which has been used throughout history to relieve pain and accelerate wound healing in traditional medicine and has recently been associated with colon cancer prevention and proposed as a potent anticancer drug. Their results suggest that LbL thin films are a promising matrix for incorporating curcumin, especially for drug delivery applications.

De Cock and co-workers used the LbL technique for loading fibroblast growth factor and heparin onto decellularized porcine aortic heart valve leaflets [55], which are common devices for human valve replacement therapy. Growth factor release under physiological conditions was sustained over 4 days while preserving the biological activity of the released growth factor. Cao and He successfully deposited multilayers of poly(acrylic acid)/prodrug onto silicon substrates via hydrogen bond-driven LbL assembly [56]. Bioactivity of the prodrug and its released free drug were studied with macrophages *in vitro*, where inhibition of nitric oxide production was observed, suggesting the desired anti-inflammatory effect was achieved.

Whereas the above approaches typically rely on diffusive transport as the mechanism of drug release, more advanced strategies depending on degradation of film components have also been proposed. Hammond and co-workers reported on LbL films for small molecule delivery capable of hydrolytic top-down film degradation, linear release profiles and programmable release kinetics through facile aqueous manufacturing (**Figure 3**) [57]. The LbL films were assembled with poly(β-amino esters) as the degradable polycations and poly(carboxymethyl-β-cyclodextrin) complexed with a small molecule as the anionic supramolecular complex. Charged cyclodextrin polymers are essential for the accommodation of cyclodextrin-drug complexes in stable fashion. These surface-eroding films are capable of drug release from within the cyclodextrin carrier without altering drug activity. Hammond and co-workers expect that this strategy would lead to the development of nanomedicine coatings for applications in personalized medicine, transdermal delivery, medical devices, nanoparticulate carriers and prosthetic implants. The same research group further investigated hydrophobic effects of polyelectrolytes on destabilization and release dynamics of degradable LbL films [58], which are determined by a complex balance between hydrophobic composition, charge density and stability of electrostatic ion pairs. The data obtained showed a correlation between the octanol/water partition coefficient and sustained release profiles that can be useful in designing custom drug delivery coatings.

Other drug delivery systems have targeted release profiles that vary with environmental stimuli, such as pH or temperature changes. For example, Anzai and co-workers prepared LbL films containing insulin and demonstrated the pH-triggered release of insulin [59]. The insulin-containing LbL films were assembled through alternate adsorption of insulin and poly(vinyl sulfate), poly(acrylic acid), or dextran sulfate in acidic solutions. Exposure of the prepared films to weakly acidic or neutral solutions induced release of insulin, owing to a loss of electrostatic forces of attraction between the insulin and polyanions. These LbL films were confirmed to be satisfactorily stable even in the presence of a digestive enzyme (pepsin) at pH 1.4. The latter result suggests the potential use of this system for future application in the oral delivery of insulin.

Finally, research to exploit the multilayer and inherently composite nature of LbL films to incorporate multiple drugs for coordinated release has also been initiated. Hammond and co-workers proposed a multiagent-delivery nanolayer that would make possible the co-delivery of different types of drug, such as charged macromolecules and uncharged, small hydrophobic drugs from a single LbL film [60]. A charged block copolymer micelle as a carrier was integrated with neutral hydrophobic drugs within the polyelectrolyte LbL films. Release of both therapeutic polysaccharides (heparin and dextran sulfate) and hydrophobic drugs (diclofenac and paclitaxel) under physiological conditions was through the hydrolytic degradation of a poly(β-amino ester) as the assembling film component. In addition, they reported that use of the spray-LbL method minimized drug loss during fabrication, increasing efficiency. Such practical development would bridge the barriers to the LbL films and direct application towards biomedical implants and devices.

4. Layer-by-layer films for delivery of biopolymers

Drugs for biomedical applications are not limited to low-molecular-mass analogues. Biopolymers including DNA, RNA, poly/oligopeptides and proteins often have more important roles. For example, localized intracellular controlled release of nucleic acid therapeutics would be an effective route to overcome the extracellular barriers that plague gene therapy. Reineke and co-workers demonstrated the LbL assembly for *in vitro* controlled release of plasmid DNA where cationic poly(L-tartaramidopentaethylenetetramine)

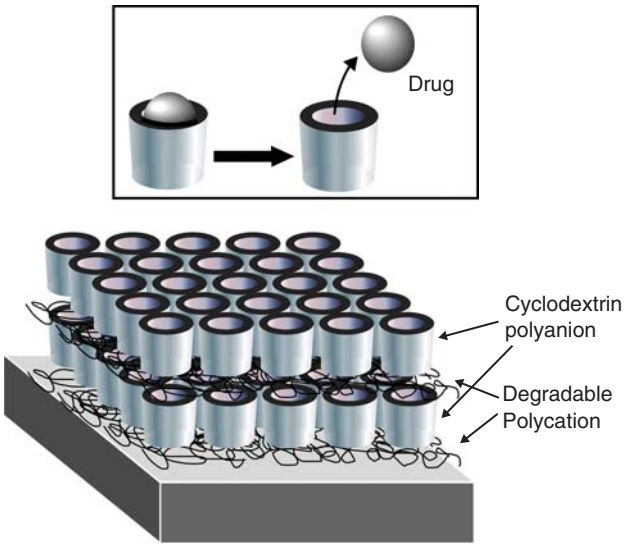

Figure 3. A layer-by-layer film made from cyclodextrin polyanion and degradable polycation for drug release.

and anionic plasmid DNA were used as assembling components [61]. Green fluorescent protein expression in HeLa cells indicated that ~ 20% of the cells showed positive expression up to 11 days, demonstrating the potential for such a system to be engineered for localized delivery of nucleic acids.

Interaction between zirconium and phosphate is also known as an effective interaction that can be exploited to construct layered films comprising nucleic acids, as pioneered by Mallouk and co-workers [62]. Wang *et al.* assembled multilayer films through coordination/electrostatic interactions between inorganic zirconium ion (Zr^{4+}) and phosphate groups in the backbone of the DNA chain [63]. LbL films were demonstrated using plasmid DNA to sustain the consecutive release of DNA and retain transcriptional activity. This strategy could be extended to other targets, from polynucleotides to other negatively charged biomolecules. In addition, it was shown that the application of a small negative potential induced decomposition of the film without electrochemical damage of DNA; such precise control of the DNA delivery kinetic profile under physiological conditions offers a tremendous advantage in the perspective of biomedical applications. In particular, selective/stimulated release can reduce the risk of ion biotoxicity for its real application. Other stimulation methods have been developed, as well, including the work of Volodkin *et al.* to demonstrate light-triggered delivery of DNA from designed LbL assemblies comprising gold nanoparticles, microcapsules and DNA [64]. Laser activation of the LbL film-supported capsules induced the remote release of encapsulated dextran. It is anticipated that light-triggered DNA transfection to a single cell can also be achieved by this approach.

Blacklock *et al.* reported modulation of DNA release from LbL films by utilizing both condensed and uncondensed DNA in different layers [65]. Polyplex layers induced faster DNA delivery in its condensed and non-aggregated state as released form. By contrast, the naked DNA layer showed slower DNA delivery, where the released DNA was more likely to be in an uncondensed aggregated state. Blacklock *et al.* proposed using the difference in reducing microenvironment between plasma membrane and the intracellular reducing environment in order to provide a specific stimulus for controlling the disassembly of DNA-containing LbL films fabricated with bioreducible polycations [66]. To test this concept, they assembled LbL films of plasmid DNA and reducible hyperbranched poly(amido amine) polycation on flexible stainless steel substrates. The fabricated LbL films showed higher and more prolonged transfection than control conventional LbL films *in vitro* and showed promising activity *in vivo*. The results obtained suggest that an exofacial reducing microenvironment of plasma membrane can indeed serve as a trigger in physiological conditions.

For improvement of transfection efficiency of small interfering RNAs (siRNAs), Fujimoto *et al.* fabricated LbL films of siRNA and cationic polymers, the branched or linear form of poly(ethyleneimine) (**Figure 4**) [67]. Transfection efficiency was evaluated in a system where siRNA specific for enhanced green fluorescent protein was electroporated on the electrode into human embryonic kidney cells stably transformed with the enhanced green fluorescent protein gene. The LbL film siRNA with branched poly(ethylene imine) facilitated an increase of the surface density of loaded siRNA. The LbL multilayers suppressed expression of green fluorescent protein gene more efficiently compared with the monolayer. Similarly, Chan and co-workers proposed a multilayer-mediated forward method for patterned siRNA transfection [68]. In this case, they used pH-responsive LbL films as the delivery platform, where a microcontact printing technique was applied to pattern nanoparticles of transfection reagent-siRNA complexes onto degradable multilayers. The proposed method provides an efficient and simple approach to spatially controlled siRNA delivery.

Deliveries of peptides and proteins are also important targets for research in the corresponding fields. For example, antimicrobial resistance to avoid biofilm-related implant failure is important for the treatment of flesh wounds as well as the functionalization of bandages, medical devices and implant materials. For this purpose, Shukla *et al.* studied the incorporation and release of an antimicrobial peptide, ponericin G1, from hydrolytically degradable LbL assembled thin films [69]. It was found that the film composition – in particular, the polyanion used – strongly influences the film growth and degradation properties as well as the incorporation and release properties of ponericin G1. Wang and Ji fabricated LbL films of poly(L-lysine) and hyaluronic acid at controlled pH conditions to induce exponential growth of the multilayer [70]. These exponential growth LbL films were utilized as reservoirs for loading a transactivating transcriptional factor peptide. As compared with the direct

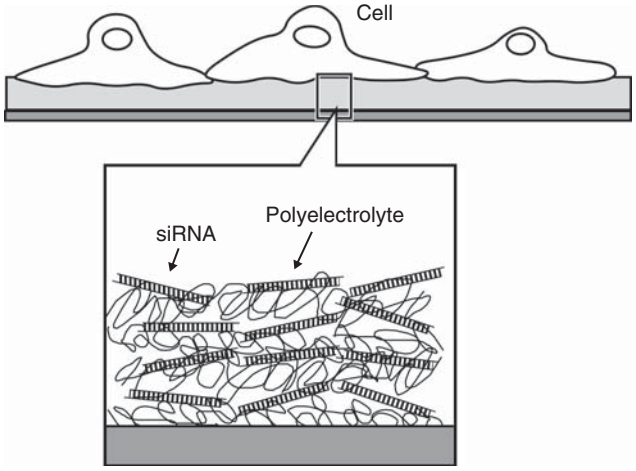

Figure 4. A layer-by-layer film of siRNA and polyelectrolyte for control of transfection efficiency to cells.
siRNA: Small interfering RNA.

LbL assembly of the transactivating transcriptional factor peptide and hyaluronic acid, greater amounts of the peptides could be loaded within the film via the post-diffusion of transactivating transcriptional factor peptide into the pH-amplified exponential growth multilayer. The post-diffusion of oligopeptide within an exponential growth multilayer can serve as an effective approach for localized transport of the peptides into cells.

Mehrotra *et al.* reported time-controlled protein release from LbL films assembled on agarose hydrogel [71], where the proteins such as lysozyme were incorporated within the degradable LbL multilayer coatings. The protein was loaded subsequent to the agarose hydrogel fabrication rather than pre-loaded directly into the hydrogel, avoiding the caustic conditions used in the templated agarose scaffold fabrication. A variety of proteins can be applied in film degradation-based controlled release without the concern or constraints that may be imposed by potential interactions between the drug and hydrogel. This approach does not require any specific chemical alterations to the LbL components. Soike *et al.* also developed nanoparticle LbL coatings for drug delivery and imaging [72]. Poly(styrene sulfonate)-functionalized poly(DL-lactic-*co*-glycolic acid) nanoparticles were loaded with model drugs, fluorescein sodium salt, bovine serum albumin and horseradish peroxidase. They proposed a four-stage mechanism of drug release as swelling of the nanoparticle, followed by saturation of the polyelectrolyte network, establishment of gradients, and osmotically-driven drug release. Crouzier *et al.* investigated use of the LbL films of a delivery reservoir for a matrix-bound recombinant human bone morphogenetic protein 2 (rhBMP-2) [73]. It was demonstrated that the amount of rhBMP-2 loaded in LbL films can be modulated significantly by varying the film thickness and/or the initial protein concentration. Applications of these LbL films in the differentiation or self-renewal of stem cells can be expected.

5. Layer-by-layer hybrid films for drug delivery

One of the most outstanding features of the LbL technique is the wide choice of layering components, which is advantageous for fabrication of hybrid structures comprising virtually any combination of materials. Hybridization of the LbL assemblies with inorganic and/or organic materials often induces significant enhancement of the whole LbL system.

As an attractive strategy of organic/inorganic hybrid LbL films, Mohammed and McShane reported fabrication of multicomponent patterned films comprising polymer/nanoparticle multilayers using conventional lithography and LbL assembly [74]. The method has potential to extend polymer surface micromachining capabilities to the construction of integrated systems by connecting discrete domains of active materials containing functional nanoparticles. The micropatterning of LbL films holds promise of utility in many biological uses, particularly in the areas of tissue engineering, biosensors and bio-interface engineering. High-resolution control over the composition and topography of surfaces will make possible more advanced studies for understanding cellular behavior. The concept of hybrid LbL films is also used for drug delivery applications. Polyoxometalate is a promising antitumor drug that has a very strong interaction with carbon nanotube surfaces. Zhao *et al.* fabricated LbL films of polyoxometalate-modified single-walled carbon nanotubes with chitosan for sustained release of polyoxometalate [75]. For better cytotoxic properties, positively charged chitosan was used to complex with negatively charged polyoxometalate-nanotube complex; because of the enzyme responsiveness and biodegradability of chitosan, these LbL-assembled films could find use as implantable drug release systems.

Hybrid LbL assemblies with mesoporous materials have also gained recent attention [76]. Some of these have been used for biological applications, including drug delivery and sensing [77,78]. Cai and co-workers assembled LbL films of chitosan/gelatin pairs where mesoporous silica nanoparticles loaded with β-estradiol are embedded for a nanoreservoir-type drug delivery system onto titanium substrates [79]. β-Estradiol release is responsible for regulating the growth of both osteoblasts and osteoclasts and, as such, the fabricated nanoreservoir structures displayed potential to maintain bone homeostasis. Thus, similar hybrid systems may find wide applications in implant technology and regenerative medicine. Tao and co-workers fabricated mesoporous silica nanotubes coated with LbL films for pH-controlled drug release [80]. The effect of pH on the interaction between polyelectrolyte multilayers as well as between hybrid composites and drug molecules alters the drug release process. The pH-responsive hybrid composites are potentially applicable for local drug delivery and cancer therapy.

Ariga and co-workers developed LbL assemblies of mesoporous silica hollow capsules with counterionic polyelectrolyte on a quartz crystal microbalance (QCM) plate through LbL assembly with the aid of silica nanoparticles (**Figure 5**) [81,82]. The evaporation rate at each step can be controlled by several factors, such as temperature and the co-adduct materials (silica particle and polymer). The evaporation behavior of water and liquid drugs surprisingly displayed a stepwise profile, including automatic release-stop sequences, even though no external stimulus was applied; this unexpected and odd behavior was observed to be reproducible. A plausible mechanism for automodulation of drug release from the mesoporous nanocompartment films is assumed to originate from the combination of two processes: evaporation from the pores and capillary penetration into the pores. Although most of the available controlled release systems perform modulation in release using some external stimulus, the mesoporous compartment film is a rare example of a stimulus-free controlled release medium, which operates in a stepwise manner with prolonged release efficiency, a feature useful for controlled release drug delivery. Recently, a similar hierarchic structure with different function was also fabricated through the LbL assembly of mesoporous carbon capsules [83]. This structure showed designable selectivity of volatile materials adsorption by impregnation of extra components for new types of sensing application.

Finally, let us introduce a potentially key natural inorganic substance for hybrid LbL structures, halloysite, as recently reported by Lvov and co-workers for drug delivery purposes. Halloysite is an aluminosilicate clay that demonstrates a predominately cylindrical geometry, uniquely characterized by a hollow core or series of voids. This material can be used as a low-cost alternative to more traditional microencapsulation systems. Halloysite microtubules have potential to encapsulate many types of material, including highly water-soluble and lipophilic materials, by exploiting either solvent replacement of bound water or chemical surface treatments. Delivery of the active reagents such as tetracycline HCl, khelline and nicotinamide adenine dinucleotide was successfully demonstrated [84]. In their separate research, Lvov and co-workers demonstrated use of halloysite nanotubules for the entrapment, storage and subsequent release of three drugs: nifedipine (anti-anginal), furosemide (anti-hypertension and diuretic) and dexamethasone (synthetic corticosteroid) [85]. Halloysite is basically biocompatible and its use in medicine for sustained drug release is feasible for dermatological and dental applications. Lvov and co-workers fabricated LbL assemblies of polyelectrolyte multilayer shells on halloysite nanotubes and achieved suppression of the release rate of dexamethasone from 7 h release for bare tubes to 30 h for polyelectrolyte-coated tubes [86]. Encapsulation of dexamethasone in the lumen of the halloysite, accomplished with the assembly of natural polymer shells, provides a new nanotube formulation for controlled release of macromolecules, including drugs, biocides and anticorrosion agents.

6. Expert opinion

In this review, an overview has been provided of recent research on LbL films for drug delivery applications. The authors believe that the combined efforts of many groups have yielded success in initial demonstrations that is sufficient for us to foresee a bright future for this technology. As the LbL method allows for a huge variety of materials and morphological and structural modifications, virtually any number of assembled structures could hypothetically be produced. Therefore, dream-like attractive systems can be proposed and experimentally demonstrated. Such trials will promote conceptual development in these fields. However, scientific research on certain aspects of LbL has matured to the point that they may enter practical stages. Drug delivery is certainly one of the most promising areas for practical applications of the LbL technology. Therefore, serious fundamental examination will be necessary to arrive at optimized systems. Although finding some difficulties in the successful examples cited above, the authors would also like to point out remaining points for further investigation from related areas.

For example, Singh and McShane have recently reported research on the reliability of the performance of LbL assemblies for biosensing [87]. They examined longevity of microparticle-based glucose sensors towards 1 month of continuous operation. Although luminescent LbL microspheres with glucose oxidase have been demonstrated as potential implantable sensors, their operational lifetime has been practically limited by enzyme degradation. By contrast, they reported that the longevity of these enzymatic microparticle-based sensors can be extended by the co-immobilization of glucose oxidase and catalase into the sensor matrix. Hydrogen peroxide deactivates glucose oxidase by means of hydrolytic cleavage of peptide bonds, which can be minimized by the action by catalase. This approach could overcome one of the primary obstacles to the deployment of enzymatic systems in minimally invasive continuous glucose monitoring. Such re-examination and reconsideration of the pre-existing systems with realistic data in practical uses is now necessary for the next step of LbL technology.

Similarly, one can find aspects that need reconsideration in 'hot' research areas such as for LbL capsules. There has been significant progress in LbL capsules for applications in drug delivery over the past 10 years, starting with the early developments driven by G Sukhorukov, E Donath, F Caruso, H Möhwald and co-workers [22,23,88-91]. Most of these works exploit the formation of nano/engineered polyelectrolyte shells on preformed cores with diameters of 1 – 5 μm, and one can find an excellent description of their achievements in recent reviews [92-95]. In this method, LbL shells were built on micronized drug particles, which allowed a slow drug dissolution time (up to 3 – 4 h) through adjustable capsule wall thickness (wall thickness of 20 – 50 nm). Furosemide, nifedipine, naproxen, biotin, vitamin K3 and insulin were mechanically crushed into a micropowder and used for LbL

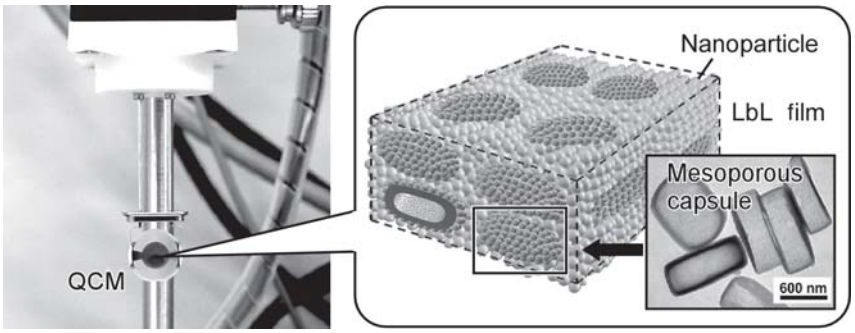

Figure 5. A layer-by-layer film of mesoporous silica capsule and polyelectrolyte on a QCM plate.
QCM: Quartz crystal microbalance.

shell assembly at a pH where they have low solubility in order to preserve the drug cores from dissolution during preparation. Typical particle sizes of such a formulation were 2 – 10 μm. In another approach, LbL microcapsules were assembled on sacrificed microcores (2 – 5 μm diameter $CaCO_3$, $MnCO_3$, or silica). Then these cores were dissolved and the empty shells were loaded with proteins or drugs through a pH-controlled capsule wall opening. Induced drug release is also possible with light-responsive capsule opening [95]. Contrary to the first case of solid drug cores, these microshells contain a relatively low amount of loaded material (1 – 5 vol%). Laser confocal microscopy allowed for the detailed studying of the structure of such microcapsules, proving the location of the loaded drugs and demonstrating their penetration into cells.

This successful development, however, did not allow for the capsule diameters to be sized on the nanometer scale. In recent publications by Lvov and co-workers [96,97], the LbL method for the production of 150 – 200 nm capsules containing low-solubility drugs was investigated in view of practical injections. The innovation was based on the use of ultrasonication to disperse better less-soluble drugs as very small particles with LbL coating. In this method, poorly water-soluble drug was dissolved in organic solvent that was miscible with water (ethanol), and drug nucleation was initiated by gradual worsening of the solution by the addition of an aqueous polyelectrolyte assisted by ultrasonication. For example, curcumin crystals of 60 – 100 nm size were obtained. LbL coating with biocompatible polyelectrolytes was used to provide a particle coating with a high surface potential and the stabilization of drug nanocolloids [98,99]. Polyelectrolyte LbL encapsulation allowed sustained drug release from nanoparticles over 10 – 20 h.

These examples provide some insight into the practical issues that should be considered in further investigations of LbL systems, although the reported examples on the LbL film systems do not give any negative impressions. Despite the great promise, we need to be extremely careful in pursuing the practical use of LbL films in realistic drug delivery systems. However, while providing these words of caution, the authors also expect that the highly versatile nature of the LbL assembly can flexibly overcome any practical difficulties encountered. The great power of LbL lies in the limitless possibilities: the layering structure, component selection and surface nature (including biocompatibility, degradability and size/dimension) can be all adjusted with this amazingly simple and versatile method. Furthermore, the inexpensive nature of this technique can also satisfy the strict economic demands of any industrialized process for large-scale manufacturing of commercial products. In addition to the great potential for biomedical uses, including drug delivery, the applicability of this methodology to other nanotechnological fabrication approaches is also anticipated. As film-type LbL nanostructures are coupled with various other fabrication processes such as advanced lithographic techniques, micro-contact printing and ink-jet printing, the realization of coupling drug delivery functions with electronic and micromachine properties may be possible.

Finally, the authors would like to add some useful information that is not fully described in this review. Although recent examples have been focused on, several excellent papers on past accomplishments [100-103] have been published, especially where broader possibilities of the LbL capsules in drug delivery are often emphasized. Combination with functional nanoparticles often leads to advanced controls of LbL drug delivery using external stimuli such as light and a magnetic field [104,105]. Although there are various examples of LbL assemblies for bio-related applications, the necessity of total consideration of mechanism aspects should be emphasized more.

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