Innovation in Layer-by-Layer Assembly

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ABSTRACT: Methods for depositing thin films are important in generating functional materials for diverse applications in a wide variety of fields. Over the last half-century, the layer-by-layer assembly of nanoscale films has received intense and growing interest. This has been fueled by innovation in the available materials and assembly technologies, as well as the film characterization techniques. In this review, we explore, discuss, and detail innovation in layer-by-layer assembly in terms of past and present developments, and we highlight how these might guide future innovation. A particular focus is on conventional and early innovations that have only recently regained interest in the layer-by-layer field. We then review unconventional assemblies that have been gaining popularity, which include the use of inorganic/organic hybrid materials, cells and tissues, stereocomplexation, patterning, and dip-pen lithography, to name a few. A relatively recent innovation is the use of layer-by-layer assembly materials and techniques to assemble films in a single continuous step. We name this “quasi” layer-by-layer assembly, and discuss the impacts and innovations surrounding this approach. Finally, the application of characterization methods to monitor and evaluate layer-by-layer assembly is discussed, as innovation in this area is often overlooked, but is essential for development of the field. While we intend for this review to be easily accessible and act as a guide to researchers new to layer-by-layer assembly, we also believe it will provide insight to current researchers in the field and help guide future developments and innovation.

1 INTRODUCTION

Thin films and coatings are a key feature of functional materials, as they often control and dictate interactions with the surrounding environment. For example, engineered interfaces can increase separation performance in capillary

electrophoresis or improve cell adherence to scaffolds for tissue engineering. Sequentially constructing thin films allows for sub-nanometer control over film thickness, while also providing highly-defined control over other physicochemical properties. Preparation methods for sequentially depositing materials of interest, or “layer-by-layer (LbL) assembly,” have

historically been developed or adapted by researchers with very different backgrounds, for specific use in their field.¹ Often, this occurs without the knowledge of similar or existing technologies in other fields. For example, as Decher discusses in the introduction to a recent book,² when LbL assembly was (re)discovered in the 1990's there were other examples of similar work from preceding decades published in other fields. Some of these studies, such as the use of an automated robotic dipping machine,³ could have facilitated and accelerated the development of LbL assembly technologies, but researchers were simply unaware of it at the time. As contributions from many fields have helped to advance LbL assembly, and in turn LbL assembly has advanced other fields, the boundaries between methodologies and technologies applied in such disparate areas has blurred.

1.1 Layer-by-Layer Assembly

LbL assembly is a pervasive method for coating substrates with polymers, colloids, biomolecules and even cells that offers superior control and versatility when compared to other thin film deposition techniques in certain research and industrial applications.⁴⁻⁹ Traditionally, LbL assembly was performed by sequentially adsorbing oppositely charged materials onto a substrate (through enthalpic and entropic driving forces),¹⁰ but quickly the applicability of interactions other than electrostatics gained interest^{11,12} after an initial study utilizing biotin-streptavidin interactions.¹³ LbL assembly

has also been the focus of numerous research articles, as thin films show promise in a number of major research fields currently being pursued.¹⁴ Therefore, numerous reviews focus on the different interaction forces applicable to LbL assembly, such as hydrogen bonding^{15,16} and unconventional interactions,¹² while other reviews focus on the properties of different LbL films,¹⁷⁻¹⁹ or on specific applications,¹⁷ such as separations,^{20,21} biomedicine,^{22,23} or drug delivery.²⁴⁻²⁸ Other reviews discuss specific methods for producing LbL films,²⁹ such as spraying^{30,31} or immersion,³² or on the interactions of LbL particles or films with different environments, such as biological systems,³³ or as barriers to gasses.³⁴ Here, we instead focus on the diverse technologies underpinning LbL assembly, and highlight the various unconventional and quasi-LbL approaches that may not typically be considered in the context of LbL assembly research, but which can provide valuable insight into how the field is developing (**Figure 1**). Moreover, we discuss the characterization methods related to LbL assembled films, as this has been crucial in differentiating between the benefits of different assembly technologies, and guide these developments. For example, how the use of quartz crystal microbalance (QCM) made film characterization simpler, thereby facilitating researchers to investigate and increase our understanding of the LbL assembly process.³⁵ Therefore, this review can act as a guide and reference for the diverse technologies related to LbL assembly, and the methods for characterizing the resultant materials.

Figure 1. Examples of LbL assembly technologies ranging from conventional to unconventional LbL assembly, and quasi-LbL assembly. This figure is intended to provide a general overview and is not meant to be exhaustive.

The concept of “layer-by-layer” is claimed by diverse fields where structures are built one layer at a time; however in this review we restrict “LbL assembly” to techniques that utilize discrete building-blocks below ~1 mm and which are sequentially coated onto a substrate or interface. By this definition, LbL assembly has existed since at least the mid 1960’s^{36,37} and has undergone numerous technological iterations since then. Interestingly, although planar and particulate substrates are handled and characterized differently, most technological improvements in LbL assembly have been applied to both planar and particulate substrates. The “traditional” form of LbL assembly uses diffusion-driven kinetics to promote adsorption onto the substrate. For planar substrates this is performed by simply immersing the substrate in a polymer or colloid solution followed by rinsing steps to wash off unbound material,³⁶ while for particulate substrates the process is more involved and requires dispersing the substrates in polymer solution followed by pelleting using centrifugation for the washing steps.^{38,39} Therefore, most LbL films are kinetically trapped structures due to the assembly methods and materials utilized and can be post-treated to shrink, swell, burst or reconfigure using stimuli⁴⁰ such as pH,^{41,42} heat,⁴³⁻⁴⁵ solvents,⁴⁶ mechanical forces,⁴⁷ competitive binding,⁴⁸ or salts.⁴⁹⁻⁵¹ Covalent crosslinking of layers can be used to improve stability against diverse solutions and solvents.⁵²

The general technological focus of improvement has been on reducing the deposition time and controlling the film properties for planar substrates,⁵³ and on eliminating centrifugation (as it introduces complexity and is difficult to scale-up) and reducing aggregation for particulate substrates.

Therefore, the embodiment of these technological advances has resulted in a conventional class of technologies, including the use of automated machines such as dipping robots⁵⁴ and the exploration of different approaches that do not rely solely on random diffusion for the transport of layering materials,⁶ for example using electromagnetism,⁵⁵ high-speed spinning,^{56,57} spraying,⁵⁸ and fluidic and vacuum based assembly.⁵⁹⁻⁶¹ Unconventional technologies such as three-dimensional (3D) printing,⁶² bioprinting,⁶³ and dip-pen nanolithography (DPN),⁶⁴ and unique interactions, such as stereocomplexation,⁶⁵ and chelation,⁶⁶ have all been integrated into the LbL assembly toolbox. Additionally, a new branch of assembly has started to emerge from the LbL field that sidesteps multilayer assembly altogether. This “quasi-LbL assembly” utilizes the conventional materials, characterization methods, and assembly techniques of LbL assembly, but instead relies on simultaneous deposition, rather than subsequent deposition to expedite the thin film or particle assembly process.

Overall, the frontiers of LbL assembly are blurring, and this review is aimed at highlighting the foundations and fringes of the LbL assembly field as they currently stand. Finally, the development and application of new techniques and assemblies must be confirmed with different characterization methods, which we discuss in the last section of this review. Characterization of thin films and understanding the phenomena governing film formation has led to new assembly techniques. Thin film characterization also allows for assembly techniques to be contrasted and compared, and can direct the application of assembled films.

Figure 2. Half a century of LbL assembly: Timeline of the progression and evolution of LbL assembly into conventional, unconventional, and “quasi” assembly. This timeline is intended to highlight the general trends of LbL assembly and is not meant to be exhaustive. Number of publications for search term: “multilayer assembly OR layer-by-layer assembly”. Search performed in Google Scholar (<https://scholar.google.com>) on August 19, 2016.

2 CONVENTIONAL LbL ASSEMBLY

Conventional LbL assembly has undergone various iterations and generally relies on equipment and methodologies common to most laboratories. In this sense, conventional LbL assembly has well-established protocols⁶⁷ with the underlying driving forces and fundamental properties of the films studied and generally understood. Numerous reviews currently exist to highlight and describe the diverse facets of conventional LbL assembly. For example, Decher's seminal review comparing LbL assembly to other thin film technologies and discussing the structure of the resulting films,⁶ Caruso's review on the nanoengineering of particle surfaces,⁶⁸ Hammond's review on how to integrate form and function into multilayered films,⁶⁹ Ariga and colleagues' review on the versatility of LbL assembly,¹⁷ Liu and coworkers' review on different template materials and architectures,⁷⁰ Borges and Mano's review on molecular driving forces used for LbL assembly,¹¹ and our recent review discussing how different assembly technologies affect the resulting multilayered films.¹ Still, the broad picture of how LbL assembly has evolved is only just starting to be untangled (**Figure 2**). In this section, we describe the technological evolution of LbL assembly over time, and discuss how methods and equipment were repurposed from other fields to be incorporated into the everyday toolbox of LbL assembly.

2.1 Immersive Assembly

LbL assembly is generally performed by manually immersing planar substrates into solutions of the layering materials followed by washing (**Figure 3**).^{30,36,71} Similarly,

particulate substrates can be layered using immersion-based approaches, however the substrates need to be collected between washing and deposition steps, which in most cases requires centrifugation.⁷² Various methodologies have been developed to expedite the layering process or reduce manual involvement by changing the adsorption kinetics or automating the layering process. Although it is simple to immerse a substrate into a layering solution and subsequently wash it, the differences between automated techniques is impressive, and ranges from crude robots driven by compressed air,^{73,74} to electrically-driven slide staining robots,^{54,75} to elegant dipping robots capable of controlling the polymer deposition using computerized feedback loops.^{76,77}

Figure 3. (A) Schematic illustration of immersive LbL assembly on a planar substrate using oppositely charged polymers, (B) the charge characteristics of the films after each deposition step, (C) the chemical formula for the polymers.

2.1.1 Manual Assembly with Planar Substrates

The “standard” conventional method for LbL assembly on planar substrates is immersive assembly, whereby the substrate is sequentially immersed into polymer solutions for deposition, with rinsing steps between the deposition steps. The earliest studies on immersive LbL assembly actually used charged particles as the layering materials, and it was noted at this time that any material with a surface charge can be used for LbL assembly as long as deposition takes place at the appropriate pH.^{3,36,71,78,79} In these early stages it was determined that LbL assembly allowed for more homogenous films to be prepared when compared with techniques such as gas deposition and nucleation deposition.³ Subsequent studies have shown that salt concentration and pH of the deposition solution, layering material concentration, immersion time, washing parameters, and other variables can cause differences in film growth.^{32,73}

Early studies used short adsorption times (1 min per layer) for material deposition due to the large size of the particles and colloids used,³⁶ while it was later determined that for standard immersive LbL assembly with polymers, the substrate should be immersed for more than 12 min for optimal layering.⁸⁰ This time requirement for deposition is one of the primary impediments to large scale high-throughput use of immersive LbL assembly. To expedite the process, dimethylformamide can be added into the layering solutions, thereby removing the need for rinsing and drying steps, as

dewetting leads to both deposition and drying.⁸¹ Additionally, dewetting LbL assembly allows for the deposition of materials not conducive to LbL assembly, such as branched SnO₂ nanowires with a low surface charge and small contact area. Similarly, having a biphasic solution of immiscible liquids with the coating liquid on top, allows for low volume coating of large planar substrates during immersion.⁸² Alternatively, deposition from alternating polar and non-polar solvents can be used to control the film morphology and thickness.⁸³ Finally, a different approach to expedite manual immersive assembly is to use a magnetic stirrer bar to mix the polymer solution during layering, which greatly speeds up the assembly kinetics (**Figure 4**).⁸⁴ From these studies it becomes obvious that slight physical agitation of the layering solution can have a significant influence on the time required and type of films formed.

Figure 4. Schematic illustration of expedited immersive assembly on a planar substrate using a magnetic stirrer bar in the polymer solutions. Adapted with permission from ref 84. Copyright 2010 American Chemical Society.

2.1.2 Manual Assembly on Particulate Substrates

Immersive LbL assembly on particulate substrates conventionally requires a separation step between the deposition and washing steps, and generally this is performed by centrifugation for solid particulate substrates (**Figure 5**).^{38,39,72,85,86} In terms of dealing with liquid substrates,

creaming/skimming cycles can be used to essentially invert centrifugation/washing as emulsions are usually lighter than water and float to the surface of the polymer solution rather than sinking.^{87,88} Centrifugation can also be used to speed up the creaming process,^{89,90} or instead lighter emulsions can be used.⁹¹ The major driving force behind the development of novel techniques for immersive LbL assembly on particulate substrates deals with attempts to avoid centrifugation because it can lead to aggregation and can be difficult to automate. For example, one approach uses solvent exchange steps between the layer deposition and centrifugation steps to reduce the aggregation of small particulate substrates during washing.⁹²

Figure 5. Schematic illustration of immersive assembly on particulate substrates using centrifugation in between washing steps. Reproduced with permission from ref 33. Copyright 2013 American Chemical Society.

A typical requirement for particulate LbL assembly is the use of a highly concentrated polymer solution that contains

orders of magnitude more coating material than what is actually required to coat the particles.^{72,93} However, by adding exact amounts of saturating polymer, the need for washing steps can be eliminated. This can lead to aggregation if the zeta-potential is not monitored closely,⁹⁴ while the use of sonication during layer deposition can also help avoid aggregation when using this approach, known as the saturation method.⁹⁵⁻⁹⁸ Mixing and sonication reduce aggregation when coating hydrophobic solid⁹⁹⁻¹⁰² or liquid substrates^{103,104} using immersive LbL assembly. These cases highlight that even though centrifugation is common, it is not strictly necessary, however avoiding it requires special consideration on a case-by-case basis.

2.1.3 Robotic and Automated Immersive Assembly on Planar Substrates

Immersive LbL assembly has undergone numerous iterations of automation.^{3,54,73-76,105-108} An elegant form of automation is the use of a QCM crystal as a substrate, which can allow for control over layering using a computer monitored feedback loop.^{76,107} This machine regulates layering based on the mass adsorbed, which is in contrast to the other automated dipping machines reported below, which use fixed immersion times for layering. For fixed immersion time machines, a computer programmed automated slide stainer can be used. This allows for automation and agitation during washing steps, and the washing solution can be constantly replenished.^{54,75} A particularly useful step in robotic immersive assembly combines machines with stirred solutions. This commercially available dipping robot has a slide holder that can rotate, allowing for expedited deposition times.^{73,74} A novel take on

automated layering uses an automated pipetting robot to pipette solutions into multiwell plates, allowing for a wide variety of variables such as incubation time, pH, ionic strength, etc., to be investigated simultaneously at relatively high-throughput.¹⁰⁹

Flexible planar substrates can also be used in roll-to-roll immersive assembly, which allows for rapid layering of large substrates, such as poly(ethylene terephthalate) (PET) (**Figure 6**).¹⁰⁵ For layering, the PET is rolled through the polycation solution, followed by rinsing solution three times, and the polyanion solution, after which the process is repeated. The immersion time and rolling speed influence the film properties. Additionally, the drying conditions and wettability need to be optimized for roll-to-roll layering to produce films with similar characteristics to standard immersive assembly.¹¹⁰ Roll-to-roll has an added benefit of being easily scalable and industrially used, making it particularly relevant for the scale-up of LbL assembly.

2.1.4 Automated Immersive Assembly on Non-Planar Substrates

For automated immersive layering, the substrates do not have to be planar. A computer-controlled custom-built immersion machine capable of depositing ~1000 layers of charged colloids was used to coat mounted and unmounted large particulate substrates (~100 μm in diameter).³ Another method for automated immersive assembly on particulate substrates uses agarose to group collections of particles into a unified planar substrate, allowing robotic dipping (**Figure 7**).¹⁰⁶ An interesting approach to non-planar automated

layering used a substrate with pores in it and applied pressure to fill the pores with polyelectrolytes and charged nanoparticles. This was significantly quicker than manual immersive assembly, and also allowed for the generation of polymer nanotubes following dissolution of the substrate.¹¹¹ A recurring theme for coating non-planar substrates with different technologies, is the use of immobilization agents to fix the substrates, yet be unobtrusive during layering.

Figure 6. Schematic illustration of automated roll-to-roll immersive assembly on flexible substrates using polymer solutions. (A) Movement through positively charged polymer and washing solutions, and (B) movement through negatively charged polymer and washing solutions. Reproduced with permission from ref 105. Copyright 2005 IOP Publishing.

Figure 7. Schematic illustration of automated immersive assembly on particulate substrates immobilized in agarose. (a) Immersion into positively charged polymer solution, (b) immersion into negatively charged polymer solution, and (c) the resultant coated particles. Reproduced with permission from ref 106. Copyright 2013 Wiley.

2.2 Spin Assembly

The common and industrially relevant coating technique of using a spinning substrate to facilitate coating and drying, namely “spin coating”, was one of the first technologies to be applied for LbL assembly.^{57,112} Although spin drying after immersive LbL assembly can be used,⁵⁶ the majority of spin LbL assembly is performed by either casting the solution onto a spinning substrate,¹¹³ or by casting the solution onto a stationary substrate that is then spun.¹¹⁴ Spin assembly has not undergone many technological improvements since its introduction to LbL assembly, due to its ease of use and presence in industry, with associated equipment developed over decades.

2.2.1 Standard Spin LbL Assembly

For polymers, spin coating deposits thinner films than immersive coating,^{115,116} however for colloids, spin coating

deposits thicker films.¹¹⁴ These properties arise because the spinning process results in more homogenous films due to electrostatic interactions, centrifugal and viscous forces, and air shear. These forces also allow for polymer films to be highly ordered with specific layer interfaces.^{117,118} For depositing colloids, these forces lead to a monolayer of colloids, while standard immersive LbL assembly often leads to less than a monolayer, i.e., the substrate is not fully coated by the particles (e.g., <30% coverage has been reported when immersing PEI-modified substrates into dispersions of colloidal gold).^{114,119} Spin assembly therefore allows for transparent or oriented LbL films to be prepared from materials that would yield opaque or disordered films using other assembly methods.^{120,121} However, a common issue with spin assembly is that the films can be thicker where the solution was cast, in comparison to the edges of the substrate. This was also observed for higher ionic strengths of polymer solution, and separately lower spin speeds.^{113,122} Spin assembly is easy to automate by integrating injection systems with rotating substrates (**Figure 8**),¹²¹ and is therefore of relevance to different applications.

Figure 8. Schematic illustration of spin assembly using an automatic injection system. Adapted with permission from ref 121. Copyright 2009 American Institute of Physics.

2.2.2 High-Gravity Spin Assembly

A unique approach to spin assembly utilizes the rotation of polymer or colloid solutions, with the substrate parallel to the axis of rotation compared with perpendicular to the axis of rotation for standard spin assembly (**Figure 9**).¹²³ This process generates high centrifugal forces down onto the planar or non-planar substrate, and is therefore termed “high-gravity” LbL assembly.¹²⁴ These high forces result in improved film assembly, especially at low polymer concentrations. Similar to standard spin assembly, the deposition of colloids and micelles is quicker and more uniform using high-gravity spin assembly.¹²⁵ Polymer combinations that grow exponentially using immersive LbL assembly, grow linearly under “high-gravity” assembly.¹²⁶ With long enough assembly times, complete desorption of polymers can be engineered with “high-gravity,” rather than the partial desorption seen with many LbL techniques.¹²⁷ An issue with high-gravity spin assembly is the requirement for the creation of a custom spinning machine, in comparison to standard spin assembly (with horizontal substrates) machines that are relatively common in research and industry settings.

Figure 9. Schematic illustration of high-gravity spin assembly using inorganic and polymer solutions. Reproduced with permission from ref 123. Copyright 2012 American Chemical Society.

2.3 Spray Assembly

Coating materials with a spray or aerosol is ubiquitous in everyday life, and is also common in industry and research. Spraying has been used in different aspects of LbL film assembly, from drying aligned films¹²⁸ to coating substrates with multilayer films.¹²⁹ In terms of coating large substrates, spray assembly⁵⁸ approaches an industrial level far surpassing other LbL assembly methods other than roll-to-toll.^{30,130} In fact, due to the general presence of spray machines, some researchers have even performed LbL assembly on a whole car using a standard drive-in car wash (**Figure 10**).^{14,31,130} Like most other technologies for LbL assembly, automation and integration with existing technologies has played a crucial role in the development of spray assembly.

Figure 10. Spray LbL assembly on a car using a custom-modified car wash. Reproduced with permission from ref 14. Copyright 2015 Chemical Society of Japan.

2.3.1 Manual Spray Assembly

Spray assembly piqued the interest of the LbL community following a systematic comparison of conventional dipping and sequential spraying for poly(styrene sulfonate)/poly(allylamine hydrochloride) (PSS/PAH) multilayers, which demonstrated that similar film quality could be achieved much quicker using spray assembly.¹²⁹ In general, films are fabricated via spraying polymer solutions onto a substrate and spraying water on the substrate to remove excess unbound polymer (**Figure 11**).^{58,129} The morphology, chemical composition, and membrane properties can be tailored, based on the polymer concentration, spray duration, flow rate, resting duration, whether the solution is sprayed vertically or horizontally, and whether or not the substrate is washed and for how long.^{58,129,131-134} Although these approaches can seem harsh, thermodynamically unstable but kinetically trapped systems, such as liposomes, can remain intact through the deposition process, although some flattening

can occur.¹³⁵ When preparing conformal coatings of 3-Dimensional (3D), non-planar substrates with spraying, vacuum can be used to speed up waiting times between spraying and washing.¹³⁶ Spray assembly also allows larger non-planar substrates such as plastic shapes to be coated, and can even be used for the assembly of metallic films without any need for electrodeposition.¹³⁷ Spray assembly has continued to gain momentum in terms of technological innovations, which has led to novel integrations of existing techniques.

Figure 11. Schematic illustration of manual spray assembly using polymer solutions. Reproduced with permission from ref 129. Copyright 2005 American Chemical Society.

2.3.2 Spin-Spray Assembly

Spray assembly has been combined with other techniques to address some of the challenges associated with spraying. For example, a disadvantage of spray LbL assembly is that gravity draining and nozzle shape-effects can lead to inhomogeneity in the films.¹³⁸ However, these issues can largely be solved by combining slow rotation with spray assembly (**Figure 12**).^{131,132,139-142} Spray assembly can be combined with standard spin assembly to greatly reduce material waste, also allowing for assembly solution concentrations 10–50 times less than those used in immersive assembly.^{132,140} Spinning while spraying can also allow biomolecules, like proteins, to attain

different secondary structures than those generated using standard spray assembly.¹⁴³ Moreover, rotating sample holders are relatively easy to engineer, allowing for spin-spray assembly to be easily automated.¹⁴⁴

Figure 12. Schematic illustration of spin-spray assembly using different solutions. Reproduced with permission from ref 142. Copyright 2015 American Chemical Society.

2.3.3 Automated Spray Assembly

Although manual spray LbL assembly is an improvement over immersive assembly in terms of hands-on processing time, it still requires human intervention and manual work; therefore, automated spray assembly systems have been developed.¹³⁹ Automated spray assembly has been applied to coat tubular membranes by rotating the membrane during spraying.¹⁴⁵ Additionally, automated spray assembly has been

monitored and controlled by QCM, using a feedback loop to track real-time film growth.¹⁴⁶ In this instance, the spray nozzle should be moved in parallel with the substrate scanning direction to obtain a flat and uniform film, and moreover higher spray pressures result in faster film deposition. Furthermore, automated spraying has been integrated with roll-to-roll processing for the coating of industrially relevant, large substrates.¹⁴⁷ Recently, an automated and high-throughput method for coating so called “PRINT” particles was introduced (**Figure 13**).¹⁴⁸ Continuous roll-to-roll processing allows for the large-scale coating of immobilized PRINT particles, which can be removed from the planar scaffold after coating.

Figure 13. Schematic illustration of roll-to-roll spray assembly on PRINT particles using polymer solutions. Reproduced with permission from ref 148. Copyright 2013 Wiley.

2.3.4 Multilayer Particle-Generating Spray Assembly

Another use of spray assembly is to form and subsequently coat nanoparticles while the coating material is being sprayed or aerosolized. For example, surface acoustic waves can be used to atomize droplets, where the solvent evaporates from the polymer solution, leading to condensation of the polymer

into nanoparticles that are then aerosolized and coated using the same process (**Figure 14**).¹⁴⁹ The particles are then dialyzed to remove excess polymer in between deposition steps, but this process can be repeated numerous times. Alternatively, numerous polymers can be sprayed at nearly the same time to allow for a one-step LbL assembly of trilayer nanoparticles, where the layers and core are determined by the spraying order.¹⁵⁰ Spray assembly for the LbL assembly of polymers on particulate substrates is still emerging, and many spray approaches for particulate matter fall under the quasi-LbL assembly section below.

Figure 14. Schematic illustration of multilayer-generating spray assembly via the atomization of polymer solutions. (a) The piezoelectric substrate produces micrometer-sized aerosol droplets. (b) Schematic illustration of the experimental setup used to synthesize a nanocarrier comprising a single polymer. Adapted with permission from ref 149. Copyright 2011 American Chemical Society.

2.4 Fluidic Assembly

Flow-based assembly can be used to deposit multilayers using fluidic channels.^{151,152} Generally, to assemble multilayers using fluidics, polymer or washing solutions are pushed through a capillary.^{153,154} Alternatively, microchannels can be manually coated by pipetting the polymer and washing solutions onto exposed fluidic channels.¹⁵⁵ Polymer and washing solutions can also be loaded into microchannels with a pump, with the solution being removed by vacuum.¹⁵⁶ Capillary forces can also be used to pull the polymer solutions through the microfluidic channels without a pump. For example, droplets of the coating solution can be placed at the fluidic inlets, allowing capillary forces to pull the polymer into the channels, and then the substrate can be rotated at high speed to remove the solution from the channels.⁵⁹

2.4.1 Automated Fluidic Assembly

Fluidic automation is a technology with a long and rich history in research and industry, and liquid state QCM is one of the most common uses of flow LbL assembly, as it provides crucial information on layer growth in real time.¹⁵⁷ More complex microfluidic devices can be used to assemble layers on a surface using capillary flow and vacuum to fill and empty multiple channels (**Figure 15**).¹⁵⁸ In that case, the high-throughput screening of film libraries (hundreds of multilayer films) using small quantities of materials is made easy with only a droplet of material needed to fill a microchannel. Instead of vacuuming, syringe pumps can be used together with fluidic devices for fluidic assembly.¹⁵⁹

Figure 15. (a) Schematic illustrations of the automated fluidic assembly process. (b) Photograph of the actual fluidic chamber. The top device is fully treated to be hydrophilic; the bottom device is selectively treated. Scale bars are 3 mm. Adapted with permission from ref 158. Copyright 2014 American Chemical Society.

2.4.2 Vacuum assembly

Vacuum is typically combined with other fluidic methods, but by itself it can be used to form unique multilayers. Aerogels can be functionalized and coated using vacuum assembly by pouring polymer solutions over the aerogel and applying vacuum to pull the solutions through the aerogel (**Figure 16**).¹⁶⁰ Conducting polymers, biomolecules and carbon nanotubes can all be used as layering materials in this instance. Vacuum assembly can also be used to assemble

layers of reduced-graphene oxide, which is otherwise challenging to use when assembling uniform multilayers.⁶¹

Figure 16. Schematic illustration of vacuum-assisted assembly on aerogels using polymer solutions. Reproduced with permission from ref 160. Copyright 2013 Wiley.

2.4.3 Fluidic Assembly for Particulate Substrates

One method to minimize aggregation during LbL assembly on particulate substrates is to use microfluidic devices.¹⁶¹⁻¹⁶⁵ A method for coating liquid particles was developed where the coating and washing solutions are flowed perpendicular to the particle flow stream.¹⁶² Another option for fluidic layering with particles is to divert the particles so that they oscillate between the polymer and washing streams as an alternative to diverting the polymer flow stream (**Figure 17**).¹⁶⁵⁻¹⁶⁷ A more detailed layering procedure, where specific geometries are used to entrap emulsions, is used for fluidic coating with alternating layers of lipids.¹⁶³ By combining fluidics with the controlled adsorption of just enough polymer to change the surface charge, an easily scalable and rapid fluidic assembly process can be achieved.¹⁶⁸ Another fluidic assembly method relies on using a fluidized bed for layering.¹⁶⁹ In this technique, the upward force of the washing or polymer solution lifting the particles is balanced against the gravitational force sedimenting the particles, which leads to a fluidized bed. This method can be used for templates below 3 μm by altering the bed geometry, and also allows for

permeability control of the resultant films.¹⁷⁰ One of the first LbL studies in the literature used columns packed with large particles, where gravity is used to pull the coating and washing solutions through the tube, thereby building up multilayers in a facile fluidic method.³⁷ Emulsions and fluidic channels for controlling particle flow are relatively abundant, but there are not many examples for LbL assembly, possibly due to clogging issues associated with polyelectrolyte complexes.

Figure 17. (a) Schematic illustration of fluidic assembly on particulate substrates using zig-zag pillars to direct droplets back and forth between polymer solutions. (b) Magnified schematic from (a). (c) Microscopy images of the droplets moving through the coating and washing solutions. Reproduced with permission from ref 165. Copyright 2011 Royal Society of Chemistry.

2.4.4 Filtration Assembly for Particulate Substrates

Instead of complex microfluidics, fluidic LbL assembly on particulate substrates can also be accomplished using physical methods for separation (**Figure 18**).⁶⁰ In a flow-based setup, solid particles and liposomes can be pumped through a tangential flow filtration system, where the polymer rapidly dialyzes out of the fluidic channels when passing through the

filters.^{171,172} Physical membranes can also be used to separate the particulate substrates from unbound polymer, either using agarose to immobilize particles in a microfluidic channel or a filter system that blocks the particles from flowing out of a chamber.¹⁷³ Filters can be combined with mechanical agitation and even vacuum pumping to allow for centrifugation-free particulate layering.^{60,174} Vacuum is difficult to use for sensitive templates like red blood cells, however for other template particles a slight vacuum facilitates the assembly process.¹⁷⁵ The use of filters is a general LbL assembly technique, which is particularly useful for coating sensitive particles such as emulsions,¹⁷⁶ red blood cells,⁶⁰ cell islets,¹⁷⁷ or particles that can easily aggregate, such as thin CaCO_3 nanowires.¹⁷⁸ Constant stirring minimizes aggregation for robust particulate substrates.⁶⁰ Additionally, an automated feedback loops for evacuating the fluid from the reaction chamber can be utilized, which can reduce the handling time by ~60%.¹⁶⁴

Figure 18. Schematic illustration of fluidic assembly on particulate substrates using filters and mixing. Reproduced with permission from ref 60. Copyright 1999 American Chemical Society.

2.5 Electromagnetic Assembly

Electromagnetic force is a useful driver for LbL film assembly, whether in the form of electric currents or magnetic fields. Electrodeposition is a well-established technology for coating materials with metals using an applied voltage in electrolytic cells,¹⁷⁹ and has even been used to assemble¹⁸⁰ and post-modify LbL films.¹⁸¹ Directing and manipulating components with magnets is also a common technique for assembling functional materials.¹⁸² Combined, electromagnetism is a useful method for assembling multilayer films with unique properties. For example, magnetic nanoparticles can be packed into films at a higher density when assembled under a magnetic field.¹⁸³ Still, electromagnetic assembly is by far the least utilized technology for LbL assembly, although it allows for the generation of unique films to be prepared that otherwise could not be assembled in a LbL manner. Electromagnetic assembly is also heavily utilized for unconventional LbL assembly and quasi-LbL assembly, as discussed in later sections.

2.5.1 Simple Electrodeposition

Electrodeposition is a common means for depositing metals, and can be used to form bimetallic mesoporous multilayer films (**Figure 19**).¹⁸⁴ Electrodeposition of polymers, enzymes, colloids and mixed material films can also be performed, however the polarities of the electrodes are generally reversed or reduced in between deposition steps.¹⁸⁴⁻¹⁸⁸ Alternatively, by placing the substrate in between the electrodes, coating without switching electrode polarity is possible, as polymer can be loaded from either end.^{189,190} Moreover, higher voltages can be used when the substrate is in between the electrodes.¹⁹¹ For general electrodeposition on an electrode, higher voltages

lead to thicker films if the polymer is sufficiently charged, although above a certain voltage the film thickness decreases as the electrode/substrate begins to repel the previously deposited layer, leading to desorption.¹⁹² Additionally, if a film becomes too thick, a sufficient current cannot be generated and film growth terminates.¹⁹³ Therefore, careful choice of the substrate positioning and voltage is crucial for standard electrodeposition assembly.

Figure 19. Schematic illustration of simple LbL electrodeposition of all-metal mesoporous films. Reproduced with permission from ref 184. Copyright 2012 American Chemical Society.

2.5.2 Complex Electrodeposition

Electric currents can be used for more than just pulling charged polymer toward an electrode, for example the pH of the solution near the electrodes can locally change, which can promote sol-gel transitioning of materials, and therefore deposition.¹⁹³ The redox potential during electrodeposition can also be used to nucleate nanoparticles during layer growth.¹⁹⁴ Similarly, the redox conditions of the electrodes can allow for unique electrocoupled films to be generated. In one case, Cu(I) was generated *in situ* from Cu(II) to crosslink azide- and

alkyne-containing polymers under an electric current, thereby forming covalently stabilized multilayer films.¹⁹⁵ Alternatively, click groups can be engineered to be electroactive, allowing for direct electrocoupling during film growth.¹⁹⁶ Specifically, C–C bonds can be formed through coupling reactions such as Heck and Suzuki reactions using *N*-alkylcarbazole derivatives. Therefore, functional units like porphyrin, fullerenes, and fluorine can be assembled into single- or multi-component films, where single-component films are assembled using potential sweeps while multi-component assembly still requires washing steps. A similar technique can be used for switching between oxidative and reductive reactions in one-pot, allowing for wash-free electropolymerization of multilayer films (**Figure 20**).¹⁹⁷

Figure 20. (a) and (b) Schematic illustration of complex electrodeposition of redox active materials. (c) Image of actual apparatus. (d) Graph of the layering speed. Reproduced with permission from ref 197. Copyright 2013 Royal Society of Chemistry.

2.5.3 Magnetic Assembly for Orienting Planar Films

Magnets and magnetic fields, rather than electric currents, can also be used to facilitate assembly or modify multilayer

films. When layering magnetic nanoparticles, deposition under a magnetic field allows for higher density, oriented films.¹⁸³ Magnetic small molecules in a multilayer can also be oriented with an external magnetic field.¹⁹⁸ Interestingly, the orientation of the magnetic field (perpendicular vs parallel) plays a large role in the final film morphology of the assembled magnetic materials (**Figure 21**).¹⁹⁹ Specifically, parallel orientation generally leads to denser packing and greater layer thickness, and can also lead to striped films due to the magnetic field lines. When making crystals from small molecules, their magnetic moment can also be engineered to face certain directions when assembling them under parallel and perpendicular magnetic fields.²⁰⁰

Figure 21. Schematic illustration of the magnetic assembly of nanoparticles using differently aligned magnetic fields. Reproduced with permission from ref 199. Copyright 2011 American Chemical Society.

2.5.4 Magnetic Assembly for Collecting Particulate Substrates

Magnets are not restricted toward orienting assembled materials, and can instead be used to separate particulate substrates from the coating materials to avoid centrifugation.⁵⁵ For example, emulsions that cannot be pelleted by centrifugation can be loaded with magnetic nanoparticles and separated from the coating solution with a magnet.²⁰¹ Additionally, solid particulate substrates impregnated with magnetic nanoparticles can also be separated from coating solutions with a magnet.²⁰² Therefore magnets can have a useful role in the processing of multilayered materials.

3 UNCONVENTIONAL LbL ASSEMBLY

In this section, we discuss multilayer assembly techniques that are less traditional in the context of thin film deposition, denoted as “unconventional” layer-by-layer assembly, with this terminology previously used in the literature.^{12,203} We provide a non-exhaustive list of pertinent examples of how certain types of films and assembly methods that are not typically associated with LbL assembly can be leveraged to assemble multilayer films in unique ways. For example, certain multilayered films, such as coordination-driven films and cellular multilayer films, and certain methods, such as using inkjet deposition and DPN, have only recently gained traction in the LbL field and can therefore be considered “unconventional” (**Figure 2**). This section focuses on three distinct areas: the first is unconventional assemblies, where we highlight examples such as “step-by-step” and coordination-driven multilayer films that have clear similarities to conventional LbL assemblies;⁶⁶ the second is multilayer film patterning, which can be challenging to achieve for films assembled on the nanoscale;²⁰⁴ and finally we cover biological

assemblies, which are often both patterned films and unconventional assemblies.²⁰⁵ A unifying theme of unconventional LbL assembly is a move toward control over assemblies at larger and smaller scales than conventional LbL assembly, as is found in multilayer films of cells and small molecules, respectively.

3.1 Unconventional Assemblies

Early studies in multilayer assembly utilized silica particles and colloidal alumina as the layer materials,^{36,37} but when LbL assembly was revitalized in the early 1990s much of the focus was on polyelectrolytes.⁶ Today a wide range of different materials can be used to form multilayers and this toolbox continues to expand,^{11,12,14} especially with the advent of new assembly technologies.¹ As the use of LbL assembly, associated materials, and relevant techniques continue to expand, the boundaries of what constitutes LbL assembly starts to blur. In this section we discuss examples of innovation in multilayer assemblies and highlight unique properties of these systems. As mentioned above, these examples are not intended to be comprehensive or fully exhaustive, but are instead intended to highlight unconventional assemblies (e.g., metal-organic frameworks and metal-phenolic networks) and unique combinations of building blocks (e.g., inorganic-organic hybrid films, stereocomplexed materials, and polyelectrolyte complexes [PECs]).

3.1.1 Inorganic-Organic Hybrid Assemblies

Inorganic-organic hybrid multilayers can be assembled using so called “cerasomes”, which are organo-alkoxysilane

proamphiphiles, prepared under sol-gel reaction conditions, to form a liposomal membrane with a ceramic surface (**Figure 22**).²⁰⁶ The ceramic surface supports the liposomal structure and prevents fusion of vesicles, which enables multilayered assemblies to be formed. Alternatively, enzymes can be used as an organic portion, allowing for microreactors to be assembled with high-surface area.²⁰⁷

Figure 22. Schematic illustration of cerasomes prepared from inorganic and organic materials. Adapted with permission from ref 206. Copyright 2002 American Chemical Society.

Metal-organic hybrids are the constituents of numerous films and constructs,²⁰⁸ some of which can be used to construct multilayered assemblies. For example, thin films can be formed through the coordination-driven assembly of Fe(III) ions and tannic acid, resulting in so called metal-phenolic network films.²⁰⁹ These components can also be used to assemble multilayered films (**Figure 23**).^{210,211} The coordination process can either be performed in “one-step” by mixing the iron and tannic acid simultaneously, which can then be performed multiple times to form multilayers in ~10 nm increments,²⁰⁹ or it can instead be done in a “multi-step” procedure where the tannic acid is adsorbed to a surface, followed by Fe(III) ions, and the cycle repeated.²¹⁰ Interestingly, when comparing metal-polyphenol films made through one-step²⁰⁹ or multi-step²¹⁰ assembly, substantial differences can be observed, both in the nature of complexation and in their physicochemical properties such as permeability and stiffness. This demonstrates how the choice

of assembly method, even with identical material components, can be used to tune the properties and performance of the resultant films.

Figure 23. Schematic illustration of the sequential deposition of Fe(III) ions and tannic acid. Reproduced with permission from ref 210. Copyright 2014 American Chemical Society.

Other examples of complexation and coordination-driven assembly of hybrid multilayer films include alternately assembling: macromolecular ligands with europium(III) ions in solution;²¹² terpyridine-substituted polyaniline derivatives with solutions containing metal ions such as zinc(II) or cobalt(II);²¹³ tridentate hexahydroxamate ligand molecules with zirconium(IV) ions;²¹⁴ or redox-active dinuclear ruthenium complexes containing phosphonate linker groups with zirconium(IV) ions (**Figure 24**).²¹⁵ The sequential assembly of metal-organic frameworks (MOF) on substrates is also commonly referred to as “step-by-step” or LbL synthesis,⁶⁶ and has been used to control interpenetration in MOFs,²¹⁶ to selectively orient the MOF crystals,²¹⁷⁻²¹⁹ and to MOF-functionalize porous alumina membranes.²²⁰ These examples highlight the opportunities that exist at the interface between layer-by-layer assembly and the field of metal-

organic materials, such as MOFs and supramolecular coordination complexes.²⁰⁸

Figure 24. Schematic illustration of the sequential assembly of a redox-active dinuclear ruthenium complex with zirconium(IV) ions. Reproduced with permission from ref 215. Copyright 2015 Royal Society of Chemistry.

3.1.2 Multilevel and Multicomponent Assemblies

Another innovation in multilayer assembly materials is in the form of “dynamic” polymer thin films. In a recent example, polymeric complexes of PAH-methyl red (PAH-MR) were layered with the polyelectrolyte poly(acrylic acid) (PAA) to assemble nanofibrillar PAH-MR/PAA films (**Figure 25**).²²¹ When a substrate that has been layered with PAH-MR is immersed into PAA solution, the polymeric complex disassembles and PAH associates with the PAA. The acidic microenvironment created by PAA then induces the formation of nanofibrils of the dissociated MR molecules. This assembly was called “multilevel and multicomponent LbL assembly” and was also shown to work using 1-pyrenylbutyric acid (PYA). This multi-step dissociation, reorganization, and reassociation of the film components during each assembly cycle in the multilayer formation show how the judicious

choice of film components and assembly conditions can be leveraged with the dynamic nature of some building blocks to engineer new types of multilayered films.

Figure 25. Schematic illustration of multilevel and multicomponent assemblies. (a) PAH-MR complexes are layered on a substrate. (b) Upon addition of PAA, PAH-MR disassembles. (c) PAH reassembles with PAA, and (d) MR molecules self-assemble into nanofibrils within the PAH/PAA film where PAA provides an acidic microenvironment. Reproduced with permission from ref 221. Copyright 2015 American Chemical Society.

Unconventional multilayered films can also be made through sequential polymerization. In one example, called “molecular layer-by-layer”, a diamine and a triacid chloride were sequentially deposited onto a substrate to build highly crosslinked and dense networks (**Figure 26**).²²² Compared to standard interfacial polymerization, LbL assembly resulted in defined multilayers with greater control over thickness, topology, and local chemical composition that was controllable at monomer length-scales. For example, the topology and local chemical composition can be controlled by the type and sequence of monomers used during each

assembly step and the thickness can be controlled by the number of deposition cycles. These benefits enabled this technique to generate water desalination membranes with enhanced performance (compared to membranes fabricated with standard interfacial polymerization).²²³

Figure 26. Schematic illustrations comparing (a) “molecular layer-by-layer” (mLbL) assembly and (b) “interfacial polymerization” (IP). Reproduced with permission from ref 222.

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3.1.3 Stereocomplexed LbL Assemblies

A rarely studied, yet interesting driving force for unconventional assemblies is stereocomplexation. Stereocomplexation occurs when the interaction between polymers with different tacticities prevails over the interaction between polymers with the same tacticity. A well-known example is the stereocomplexation of isotactic (it) and syndiotactic (st) poly(methyl methacrylate) (PMMA).²²⁴ This nonionic interaction between it-PMMA and st-PMMA can be

a driving force for LbL assemblies.²²⁵ When QCM substrates are immersed in it-PMMA and st-PMMA acetonitrile solutions alternatively, the molar ratio of st-PMMA and it-PMMA in the film will be 2, suggesting a 2:1 stoichiometry for the stereocomplex.²²⁶ Similar to enzymes containing elaborately well-defined molecular spaces that exploit weak non-covalent interactions to synthesize chiral biopolymers, porous it-PMMA films can be a good template matrix for synthesizing st-PMAA.²²⁷⁻²²⁹ The regular molecular spaces allow for highly stereoselective polymerization of MAA to st-PMAA with isotacticities >94%, and a molecular weight twice that of the template it-PMMA, meaning that both the molecular weight and tacticity can be translated from the porous matrix (it-PMMA) onto the internally synthesized polymer (st-PMAA).

Stereocomplexation can even take place with chemically dissimilar polymers. It-PMMA and structurally similar st-PMAA can also form stereocomplexes. Interestingly, st-PMAA can be 100% selectively extracted in an aqueous alkaline solution from a multilayer film of it-PMMA/st-PMAA due to solubility differences.²³⁰ After extraction, st-PMAA can be re-infiltrated using immersive assembly, while atactic-PMAA cannot infiltrate the films, indicating that the porous films recognize the tacticity of PMAA. Moreover, the solvent effects on the incorporation of st-PMAA into it-PMMA have been investigated in detail.²³¹ For example, when a mixed solvent (acetonitrile/water) is used, the crystallinity of it-PMMA increases as the water content increases,²³² and this crystallization reduces the amount of st-PMAA incorporation.²³³ Hollow crystalline LbL capsules (**Figure 27**)

can be prepared with the stereocomplex (it-PMMA/st-PMMA)₁₀.²³⁴

Figure 27. Schematic illustration of capsule preparation through PMMA stereocomplexation LbL assembly. Reproduced with permission from ref 234. Copyright 2006 Wiley.

Enantiomeric poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA) can also form stereocomplexes, in which the left- and right-handed 3₁ helices pack side by side via van der Waals interactions,⁶⁵ which can be harnessed for LbL assembly.^{235,236} The alkaline hydrolysis of the stereocomplex PLLA/PDLA LbL films was quantitatively investigated using QCM,²³⁷ and it was found that the stereocomplex is more readily hydrolyzed compared to crystalline polylactic acids (PLAs). This hydrolytic rate is more similar to that of amorphous PLAs, allowing for tailored degradation profiles depending on the film architecture. Hollow PLLA/PDLA multilayer capsules can also be prepared using stereocomplexation LbL assembly.²³⁸ Stereocomplexation is therefore a useful method for forming films on planar and particulate substrates.

3.1.4 LbL Assemblies of Polymer Complexes

An alternative approach to LbL assembly is the sequential deposition of PECs into films, yielding thicker films than those composed of the free polymers. For example, films for organic electronics can be made from an aqueous suspension of poly(3,4-ethylenedioxythiophene)-PSS (PEDOT-PSS), a

zwitterionic but overall negatively charged complex.²³⁹ This shows that LbL assembly can be performed with materials of similar charge. Therefore, the 2-in-1 LbL assembly method allows for the *successive* deposition of the same solution containing pre-formed polyanion/polycation complexes or a single zwitterionic species (**Figure 28**).²³⁹ Four different systems (PEDOT-PSS, bPEI-PSS (branched poly(ethylene imine)-PSS), poly(diallyldimethylammonium chloride) (PDADMAC)-PSS, and PAH-PSS), were investigated, demonstrating the generality of this approach. (Note) that some studies do not use the counter ion chloride in PDADMAC, but the polymer will be abbreviated ‘PDADMAC’ in this review for simplicity. Notably, the film morphology was different than it was for films of the same components prepared using conventional immersive LbL assembly. It was suggested that this behavior depends on the pre-formed PECs, and whether they are more solid-like (leading to rather rough films) or more liquid-like (generating smooth films). Similar to some quasi-LbL assembly methods mentioned later (see section 4), local zwitterionic charge distributions are necessary for film formation.

Figure 28. Schematic illustration of the 2-in-1 film assembly method using a single solution containing polycation/polyanion complexes. Reproduced with permission from ref 239. Copyright 2012 American Chemical Society.

3.2 Unconventional LbL Film Assembly and Patterning

The patterning of films, or “lithography”, is both the foundation of the relatively high-tech semiconductor industry,²⁴⁰ and relatively low-tech book-printing industry.²⁴¹ However, many of the methods used for wafer processing are based on the high-resolution deposition of inorganic materials, namely conductors consisting of metals and insulators such as silicon dioxide. Alternatively, traditional ink-based lithography generally used small molecule inks with low spatial resolution. Therefore, significant innovation and inspiration from older techniques of typographical lithography was required to move patterning from the inorganic regime most closely associated with semiconductors, to the organic regime of polymer thin films commonly encountered with LbL assembly.^{203,242} By judiciously matching lithographic techniques with selected materials, LbL films can be successfully patterned in a controlled manner.⁶⁹

3.2.1 Additive Lithography

One of the early innovations in LbL patterning was the use of microcontact printing to chemically define areas of enhanced and reduced deposition.^{75,243,244} In microcontact printing, an elastomeric mold is made and coated to create a stamp that can transfer a pattern onto a substrate.²⁴⁵ This elastomeric mold in turn is made from a silicon wafer mold that has been patterned through standard lithography techniques, a process commonly called soft lithography.²⁴⁶ In this way the power of lithographic techniques associated with the semiconductor industry can be used to pattern (through a middle-step using an elastomeric mold) substrates before or

after LbL assembly. Typical feature sizes are on the micrometer length scale, although patterns with features down to few hundred nanometers can also be achieved, and some lithographic techniques even offer sub-nanometer resolution, such as through the use of nanoshaving or nanografting.^{247,248}

In one example of microcontact printing for patterned LbL assembly, gold-coated silicon wafers were patterned with 16-mercaptohexadecanoic acid and subsequently immersed in (11-mercaptopundecyl)-tri(ethylene glycol).²⁴³ This created a self-assembled monolayer of spatially confined negatively charged patches (COOH/COO⁻ terminated) to allow for directed LbL film assembly of PDADMAC and PSS (**Figure 29**). However, this behavior is strongly material-dependent and sometimes counterintuitive, e.g., for weak polyelectrolytes the deposition pattern can change, or even reverse, depending on pH.²⁴⁹ Microcontact printing can also be used to assemble patterned multilayer films, both side-by-side (laterally)²⁵⁰ and on top of each other (vertically)²⁵¹. The multilayer film can also be assembled directly onto the patterned elastomeric stamp and then transferred onto a substrate in its entirety through microcontact printing.²⁵² Moreover, microcontact patterned LbL films can be further functionalized with LbL capsules, resulting in complex patterned films.²⁵³ Therefore, microcontact printing has significant use for the pre- and post-modification of LbL films.

Another additive lithographic technique that has recently gained attention for the assembly of LbL films is DPN. DPN was developed using an atomic force microscope (AFM) cantilever tip to transfer alkanethiols onto a gold surface.⁶⁴ The tip is dipped into a solution containing alkanethiols and is then brought into close proximity of the substrate, which induces

deposition of the molecules onto the surface through capillary transport. This method has been used to create patterned LbL films, both by patterning prior to layer-by-layer assembly, but also by using DPN to assemble the layer-by-layer films themselves.²⁵⁴ DPN can be used to pattern regions prior to layer deposition.²⁵⁴ After patterning a gold substrate with mercaptohexadecanoic acid, the remaining unpatterned regions were passivated with molecules such as octadecanethiol, 16-mercapto-1-hexadecanol or (11-mercaptoundecyl)-tri(ethylene glycol). LbL assembly with PDADMAC/PSS was then performed through immersive assembly with the multilayer film forming on top of the mercaptohexadecanoic acid pattern. In a more recent example, DPN was used to directly pattern multiple lamellar layers of palladium alkanethiolates onto a substrate (**Figure 30**).²⁵⁵ After deposition, the inorganic backbone of Pd-S acts as a stabilizer, effectively crosslinking the layer through Pd-Pd interactions. This forms a relatively rigid layer that subsequent layers can be deposited onto. The fabricated structure is based on the LbL assembly of the lamella formed by the palladium alkanethiolate, and is therefore different to conventional LbL assembly, as it does not involve deposition of alternating species. Because of the sub-nanometer accuracy of AFM, this method allows for high-resolution patterning in x, y, and z. Similarly, mixed and unmixed solutions of oppositely charged polyelectrolytes can be used as inks with an AFM to assemble high-spatial resolution multilayer constructs.²⁵⁶ Although there are some requirements for “inks” in DPN, these results provide inspiration for the development of new types of patterned LbL assembled structures.

Figure 29. Schematic illustration of the patterning of a substrate before LbL assembly to create patterned multilayers. Adapted with permission from ref 243. Copyright 1995 American Chemical Society.

Figure 30. Schematic illustration of DPN for the site-specific deposition of palladium octylthiolate lamellar layers. Reproduced with permission from ref ²⁵⁵. Copyright 2013 American Chemical Society.

Inkjet deposition is another common additive technique for assembling multilayer films (**Figure 31**). For example, a standard photo printer capable of printing on CDs can be adapted for LbL deposition by modifying the CD holder to hold other substrates and by filling the ink cartridges with aqueous solutions of coating material.⁶² Patterns can then be designed using standard graphic design software and sent to the printer for pattern printing, with each layering cartridge corresponding to a separate color in the software. Essentially,

this method allows for pre-programmed patterning of LbL assemblies without multiple washing steps due to the low droplet volumes (e.g., picoliter). Furthermore, inkjet printing can be used for stereocomplexed polymers (PLLA and PDLA) to allow for drug loaded multilayer films to be prepared with drugs that would not normally be soluble.²⁵⁷ Generally, standard inkjet printers are designed for typical paper sizes and the possibility of patterning multicomponent nanocomposites in three dimensions allows for complex assemblies that are difficult to engineer using other methods, such as the inkjet multilayer assembly of polymer nanotubes using porous membranes as a template.²⁵⁸

Figure 31. Schematic illustration of LbL assembly using inkjet printing. Reproduced with permission from ref 62. Copyright 2010 American Chemical Society.

3.2.2 Subtractive Lithography

Patterning through selective removal of portions of the multilayer film can be performed through conventional photolithographic methods using photoresists and/or metal masks (**Figure 32**),^{242,259} although reversible patterning with masks can be performed without material loss if photoswitchable materials are incorporated.²⁶⁰ LbL films can be assembled on top of already patterned photoresists, after which portions can be removed through lift-off (dissolution of the photoresist).²⁶¹ By combining exposed with unexposed regions and sequential development, LbL films that are partly

attached to the substrate and partly freestanding, like arched bridges, can be engineered to form thin cantilevers.²⁶² Alternatively, patterned areas can have unique material properties that can be harnessed for LbL assembly, for example patterned indium tin oxide (ITO) substrates for electrodeposition assembly.²⁶³ Stamps can also be used for a combination of additive and subtractive lithography where the stamp is used to remove a portion of a LbL film, thereby patterning the film and generating a second pattern on the stamp itself.²⁶⁴

Figure 32. Schematic illustration of patterning multilayered films using (a) lift-off, (b) metal-mask, and (c) a combination of lift-off and metal-mask methods. (d) Patterned line of multilayer silica nanoparticles, and (e) 3D plot of the patterned structure. Adapted with permission from ref 261. Copyright 2002 American Chemical Society.

Photoresists can be deposited on top of a preassembled LbL film for patterning.²⁶¹ This can be achieved by coating the LbL film with aluminum using thermal evaporation, and subsequently spin coating the photoresist onto the aluminum.

After patterning and developing the photoresist, the partly exposed aluminum can be etched using phosphoric acid and nitric acid. The underlying LbL film is then exposed and can be etched (and thus patterned) using oxygen plasma etching. Direct-write (mask-less) lithography patterns can also be performed by “writing” into a photoresist directly with a UV laser beam.²⁶⁵

A unique example of subtractive lithography utilizes both inkjet printing and photolithography to selectively etch a pattern into a film (**Figure 33**). Hydrogen bonded multilayers of polyacrylamide and PAA assembled at pH 3 can be selectively removed by printing an aqueous solution at neutral pH over the multilayer film in a pre-designed pattern where the “printed” neutral region can be washed away to create the pattern.²⁶⁶ Using the same system, simple photolithography with a mask can be applied to selectively expose part of the polyacrylamide/PAA multilayer film doped with photoinitiator-labeled PAA. This generates free radicals upon UV exposure capable of inducing cross-linking, after which the unexposed (and therefore non-crosslinked) areas can be removed by washing with water at neutral pH.

Figure 33. Schematic illustration of subtractive patterning of preassembled multilayer films by ink jet printing of solutions different pH (left) or by photolithography (right). Adapted with

permission from ref 266. Copyright 2002 American Chemical Society.

3.3 3D Bio-Based Assemblies and Assembly Techniques

Many applications sit at the interface of unconventional assemblies and unconventional assembly techniques,²⁶⁷ with biointerfaces being obvious candidates. Cell-film interactions can be tailored based on bioactive and mechanical cues, allowing for control over cell adhesion, proliferation, and differentiation.²⁶⁸ Moreover, spatially organized films allow for the fabrication of complex systems closely mirroring the inherent complexity of nature. However, the cytotoxicity of conventional LbL materials, such as high concentrations of the polycations PEI or poly(L-lysine) (PLL), can limit the applicability of traditional polymeric LbL building blocks for biomedical applications.²⁶⁹ Comprehensive summaries of LbL films for biomedical applications can be found in several reviews.²⁷⁰⁻²⁷⁵ In the following section we focus on how literally stacking cells in a layer-by-layer fashion has inspired tissue engineering to expand from scaffold-based approaches to the fabrication of scaffold-free tissue constructs and organ printing. The bioengineering of tissue is promising for drug screening, toxicological testing, and personalized medicine, and LbL assembly will continue to play an important role in these developments.

3.3.1 LbL Assembly for Scaffold-Free Tissue Engineering

The development of cell sheet engineering, mostly inspired by the development of thermo-responsive culture surfaces in the early 1990s, gave rise to the idea of obtaining more complex 3D tissue constructs by stacking cell sheets on top of

each other (**Figure 34**).²⁷⁶ Grafting poly(*N*-isopropylacrylamide) (PNIPAM) to standard tissue culture dishes renders the surface hydrophobic and thereby allows for cell attachment and proliferation under standard culture conditions at 37 °C.^{277,278} However, decreasing the temperature below 32 °C, i.e., below the lower critical solution temperature of PNIPAM, results in hydrophilic surface characteristics that spontaneously detach the cultured cells without any enzymatic treatment, such as trypsinization, required. This mild treatment allows cells to be recovered as intact sheets, meaning that their extracellular matrix (ECM) is kept intact.²⁷⁹ By maintaining intercellular, as well as cell-to-ECM connections, improved tissue regeneration after transplanting layered smooth muscle sheets was observed when compared against injecting single cell suspensions. A primary advantage of layering cell sheets for tissue reconstruction is that no scaffold is needed, and therefore complications relating to biocompatibility and degradability of the scaffold material can be avoided. An additional benefit of 3D tissue constructs is that they can be of higher physiological relevance, as demonstrated by the simultaneous pulsation and establishment of gap junctions that can be observed for layered cardiomyocytes *in vitro*.²⁸⁰ These unique properties make layered cell sheets interesting candidates for pharmaceutical research.²⁸¹

Figure 34. Schematic illustration of tissue reconstruction using multilayers of cell sheets, where the cell sheet structures dictate the final tissue of choice. (A) Single layer sheets, (B) multilayer sheets of the same cell type, (C) multilayer sheets of different cell types, and (D) patterned sheets of different cell types. Reproduced with permission from ref 276. Copyright 2005 Elsevier.

Similar to polymeric LbL films, the properties of biological constructs can be tailored by choosing different types of cell layers, allowing researchers to mimic the complexity and hierarchical architecture of actual tissues and organs more accurately. For example, a three-layered blood vessel consisting of smooth muscle cells, fibroblasts, and endothelial cells can be engineered.²⁸² Of note, cell sheets of smooth muscle cells and fibroblasts do not need to be grown in thermoresponsive dishes but can be peeled off after 30 days of culture and wrapped around tubular supports. After maturation, the tubular supports can be removed and the endothelial cells can then be seeded in the lumen to obtain engineered blood vessels.²⁸² However, a limitation of stacking cell sheets on top of each other is the reliance on passive diffusion for the delivery of nutrients and waste removal. A first step toward the vascularization of thick cell constructs was made by implementing prevascular capillary-like networks into cell sheets. This allows for a rapid and easy

connection to the host vessels after transplantation.²⁸³ Resected tissue can be used as a vascular bed and overlaid with cardiac cell sheets to form vascularized 3D cardiac constructs.²⁸⁴ These, and similar works, have been summarized into a detailed protocol on how to engineer different types of cell sheets,²⁸⁵ as cell sheet engineering has serious implications for tissue regeneration.²⁸⁶

3.3.2 Thin Films as Mediating Layers between Cell Constructs

Owing to the challenges in handling fragile cell sheets, more robust alternatives to cell sheet engineering have been explored. A review on complex 3D tissue fabrication techniques can be found elsewhere.²⁸⁷ Here, we highlight how multilayer films can act as mediating layers by controlling inter-layer cell-cell communication. The first cellular/polymer multilayers were prepared in the late 1980s, where 3D tissues were engineered from collagen. Cell monolayers can be grown on a thin collagen I gel, after which a thin layer of collagen can be cast as an “adhesive” on top of the cells, and the process can be repeated to form multilayers.²⁸⁸ In the early 1990s this technique was expanded, and it was shown that sandwiching hepatocytes between collagen I layers improved the maintenance of liver-specific function and cytoskeletal organization *in vitro*.²⁸⁹ LbL films can be used as the mediating layers for multilayered cellular architectures, for example with the use of bio-compatible LbL films composed of chitosan/DNA for stable hepatocyte culturing *in vitro*.²⁹⁰ The polyelectrolyte scaffold helps to maintain liver-specific functions and cell morphology. Moreover, it can act like

intercellular “glue”, allowing for the culture of other cell layers (hepatocytes, fibroblast or endothelial cells).

Cellular cocultures arranged in microarrays can be generated using ECM proteins, such as hyaluronic acid (HA), fibronectin (FN), and collagen, combined with different cell lines, through LbL assembly.²⁹¹ HA-patterned substrates can be treated with FN, generating a cell-adherent surface in the areas free from HA. Cells can then be seeded onto those patches and the whole surface covered with collagen, which exhibits strong interactions with HA, thus making the HA pattern cell-adherent (**Figure 35**). Patterned and layered constructs employing cell/ECM protein mixtures can be made in microfluidic channels, allowing for repeated growth based on seeding additional cell layers after gel matrix shrinking that occurs during gelation.²⁹² Magnetic assembly can instead be used in combination with magnetic particle loaded liposomes adsorbed onto cell surfaces, where a coculture of hepatocytes and endothelial cells can be arranged using a magnet.²⁹³

Figure 35. Schematic illustration of the fabrication of a co-culture system using capillary force lithography and layer-by-

layer deposition. Adapted with permission from ref 291. Copyright 2006 Elsevier.

Many *in vitro* hierarchical cell manipulation techniques have been developed to mimic the cell-cell interaction mediating properties of the ECM. In some cases, 3D cellular multilayers were separated by thin films of natural ECM components, such as FN and gelatin.²⁹⁴ While FN-only films were not sufficient to provide adhesive support for the subsequent cell layer, thin films (6 nm) of FN/gelatin allowed for the creation of multilayered tissue. However, similar to the cell sheet engineering techniques mentioned above, vascularization of the construct is critical to obtain thick and functional artificial tissue. Additionally, this cell manipulation technique is rather time-intensive, as the adhesion of a new cell layer requires sufficient time (about 6 h for a layer of fibroblast cells). To overcome these limitations, a facile and one-step approach has been developed, termed the “cell-accumulation technique”,²⁹⁵ where instead of depositing a thin film on a cell monolayer, individual cells are coated with the FN/gelatin film. This leads to the formation of layered structures one day after cell seeding (**Figure 36**). Therefore, hetero-cellular tissue constructs can be prepared within 2-3 days via sequential seeding of the desired cell types, with a maximum number of 8-10 layers, as limited by the cell seeding density and nutrient supply. To allow for the fabrication of even thicker constructs, sandwich cultures of human umbilical vein endothelial cells can be assembled in layers of human dermal fibroblast cells, leading to vascularization of the construct.²⁹⁵

Figure 36. Schematic illustration of the rapid construction of 3D multilayered tissues by the cell-accumulation technique. Adapted with permission from ref 295. Copyright 2011 Wiley.

3.3.3 3D Bioprinting

The move from two-dimensional (2D) printing to 3D printing has revolutionized many areas in engineering and healthcare.²⁹⁶ With regards to tissue engineering, 3D printing facilitates the construction of complex scaffolds.²⁹⁷ The development of aqueous based systems for the direct printing of biomaterials allows for the incorporation of cells and specific proteins into a 3D matrix. Moreover, the precise spatial control over deposition has driven the development of scaffold-free tissue engineering, enabling direct printing of tissues and organ constructs. 3D bioprinting in a LbL manner can be classified into different categories based on the technology used for material deposition, namely capillary-based printing or laser-assisted bioprinting.²⁹⁸⁻³⁰⁰ A review on the technical details and comparisons between the different approaches can be found elsewhere.²⁹⁶

Like additive lithography (mentioned in the unconventional assembly techniques section), commercial printers can be modified to enable printing of 2D sheets of cells and proteins, allowing for spatial control and precise positioning into three dimensions.⁶³ Realizing the potential of thermosensitive gels (like PNIPAM) for multilayer cell printing and cell self-assembly has allowed artificial organs to be printed. For example, the computer-aided printing of natural materials (cells or matrices) can be performed to generate 3D structures.^{301,302} This has been formulated as a general approach with different printer designs, and is based on the

capability of adjacently placed cell aggregates or soft tissue fragments to fuse and form functional tissue (**Figure 37**).³⁰³ The 3D bio-printing of smooth muscle cells and fibroblast spheroids with agarose rods as a molding template—allowing for the generation of single- and double-layered vascular tubes—has also been reported.³⁰⁴ Essential for this type of tissue formation is a high cell density, meaning that tissue spheroids are ideal candidates for organ printing.³⁰³ Interestingly, the use of different types of tissue spheroids allowed for vascularization of such tissue constructs. Inkjet printing of single cells can also be accomplished after coating with FN/gelatin.³⁰⁵ For microbes, inkjet printing can be performed by creating silk nests formed from the LbL assembly of silk modified with either PLL or PGA.³⁰⁶

Figure 37. Schematic illustrations and actual images of a bioprinter. (a-d), Images of bioprinters with different nozzles. (e-h), Schematic of bioprinting continuously (e) and (f) and discretely (g) and (h). (i) Schematic of the LbL assembly process and eventual fusing of the construct. Reproduced with permission from ref 303. Copyright 2009 Elsevier.

Besides printing with commercial 2D or 3D printers, laser-based bioprinting allows for the deposition of different biological materials, including nucleic acids, proteins, and

cells by pulsing a laser through a support carrier to deposit the material onto a substrate.³⁰⁷ 3D cell patterns can therefore be obtained by sequentially depositing cells and manually spreading layers of Engelbreth-Holm-Swarm sarcoma extracts manually on top of each cell layer. Another laser-assisted approach allows for the engineering of skin tissue (dermis and epidermis) by embedding fibroblasts and keratinocytes in collagen with LbL assembly (**Figure 38**).³⁰⁰ The formed adherens and gap junctions allow for intercellular adhesion and communication and successful synthetic tissue formation.

Figure 38. Schematic illustration of laser-induced jet printing for the formation of cellular multilayers. A printed grid structure (top view and cross-sections) of fibroblast (green) and keratinocyte (red) multilayers. The entire structure has a height of ~2 mm and a base area of 10 mm². Scale bars are 500 µm. Adapted with permission from ref 300. Copyright 2012 Wiley.

3.4 Summary of Unconventional LbL Assemblies and Technologies

In summary, LbL assembly offers distinct advantages for the fabrication of thin films, and as the number of tested and

verified materials and methods develop, the opportunities rapidly increase. Much of the early work in LbL assembly aimed at understanding film formation at the molecular scale and the resultant material properties. Therefore, significant work was performed using “standard methods” with “standard polyelectrolytes;” for example, immersive assembly with PAH or PDADMAC and PSS, as performed by Decher, Hong and Schmitt in an early seminal LbL paper.⁷ Over time similar methods and materials became the standard and in a way helped define what constituted conventional LbL assembly. As the field of LbL assembly grew, neighboring fields with researchers from diverse backgrounds have increasingly contributed. This has blurred the boundaries between different fields, and today, exciting work is being performed at the interface of various technologies and methods in different disciplines. Unconventional examples highlighted herein include lithographic techniques, 3D printing and DPN, as well as dynamic films, coordination-driven assembly, and stereocomplexation to generate new types of multilayer films. Moreover, many improvements in cell culturing and tissue scaffolding have found inspiration from LbL assembly techniques. As these interdisciplinary efforts in multilayer assembly mature and the first two letters in unconventional slowly fade, new opportunities for innovation in the assembly of LbL films and structures will be found.

4 QUASI-LbL ASSEMBLY

Numerous technologies have been developed to accelerate and control LbL assembly;¹ however a particularly interesting innovation for LbL assembly has followed a different route by way of avoiding multilayer assembly altogether. During the

development of spray LbL assembly, it was noted that simultaneously spraying polyelectrolytes also resulted in thin film formation and that these films had unique properties.³⁰⁸ This is not actually LbL assembly, but more a film deposition technique inspired by, and in the spirit of LbL assembly, hence we term this “quasi-LbL” assembly. Therefore, quasi-LbL films are often composed of traditional LbL building blocks and often utilize conventional assembly technologies, but do not use a LbL approach and instead use a single deposition step for the two or more building blocks. This can result in interpenetrated assemblies, or even the spontaneous assembly of layered films.³⁰⁹ Similar to how the choice of assembly technique dictates the final film properties, LbL assembly and quasi-LbL assembly give rise to different nanoarchitectures.^{14,310} Like most other innovations in LbL assembly, the field of polyelectrolyte complexes and coacervates existed long before being integrated into the field of LbL assembly,³¹¹ however quasi-LbL assembly is not the preparation of either of these materials, rather it is the assembly of these materials into thin films and constructs.

4.1 Spray Assembly

Unlike conventional and unconventional LbL assembly, which grew off the back of immersive assembly,² quasi-LbL assembly has a large portion of its technological roots in spray LbL assembly. A handful of papers and patents on spray assembly were submitted within a narrow timeframe. The first peer-reviewed research paper reporting spray-assisted LbL assembly demonstrated that sequentially spraying polyelectrolyte solutions with intermediate rinsing steps allowed for the fabrication of multilayer films.⁵⁸ Several years

later, the simultaneous spraying of layering solutions with two parallel nozzles to coat a rotating cylinder was reported.³¹² However, the focus of this quasi-LbL assembly work was on the acid resistance and permeability toward different drugs of the films, and there was little in-depth physicochemical film characterization. In 2010, the spraying of solutions containing oppositely charged polyelectrolytes was introduced to form monodisperse spherical PECs with properties beneficial for drug delivery.³¹³ Simultaneous spray assembly is therefore one of the most versatile quasi-LbL assembly techniques and is useful for a wide variety of applications.

4.1.1 Simultaneous Spray Assembly on Planar Substrates

Challenging some traditional LbL assembly beliefs, simultaneous and continuous spray coating of poly(L-glutamic acid) (PGA) and PAH onto a vertically positioned substrate produced a uniform film around 100 nm thick after 90 s of spraying.³⁰⁸ This suggested a fundamentally different mechanism of film formation based on a non-stratified film architecture. Moreover, linear film growth over time can occur instead of an exponential increase in film thickness, which occurs for the conventional LbL assembly of PGA/PAH. The concept of using traditional LbL building blocks and depositing them in a non-LbL approach can be further generalized toward polyelectrolyte/nanoparticle, inorganic/inorganic, and even polyelectrolyte/small oligo-ion assemblies (**Figure 39**).¹⁴¹ For example, films such as PAH and sodium citrate that cannot be deposited using conventional LbL assembly, can be formed through quasi-LbL spray assembly.¹⁴¹

Figure 39. Schematic illustration of simultaneous spray assembly, and actual images of (a) Na and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, (b) PAH and PSS, (c) PAH and sodium citrate, and (d) PAH and gold nanoparticles. Reproduced with permission from ref 141. Copyright 2010 Wiley.

One of the classic LbL systems, PAH/PSS, was used to investigate the fundamental rules governing the formation of thin films through quasi-LbL spray assembly,³¹⁴ where it was found that the polymer spraying rate ratio is crucial for achieving a maximum film growth rate. Additionally, it was demonstrated that both the granular film morphology of the assembled films consisted of surface features and the resultant ~1:1 PAH/PSS ratio was not dependent on the spraying rate ratio, and almost completely independent of the ratio of sprayed PAH to PSS. However, this can be attributed to the salt-free solutions of the experiments, as negligible extrinsic charge compensation was possible due to the lack of shielding ions. Therefore, electroneutrality of the film is achieved by intrinsic charge compensation between polycations and polyanions, resulting in a 1:1 ratio of PAH/PSS in the films. It was postulated that the ideal polyelectrolyte complex for the maximum film growth rate is expected to be large and rather

neutral but with large positive and negative patches (i.e., charge fluctuations). Building on these early results, the aspect of different film morphologies (i.e., smooth and liquid-like versus rough and granular) was addressed with the assembly of polyelectrolytes and small oligo-ions.³¹⁵ Here, differences in film morphology were dependent on the interaction strength between the polyelectrolyte and small oligo-ion. For weakly interacting species (PAH/citrate), a smooth, liquid-like film morphology can be observed, similar to PAH/PGA, whereas for strongly interacting components such as PAH/sulfonated cyclodextrin and separately PAH/PSS, a granular topography was observed via AFM.

4.1.2 Spray Assembly to Form Particulate PECs

Instead of spraying onto a substrate, microparticles can be formed by spraying and quickly drying a solution containing polyelectrolytes (**Figure 40**).^{31,313} Spraying is generally achieved with a coprecipitation agent that can later be dissolved to form pores, such as nanometer-sized calcium carbonate.³¹³ Similar to conventional LbL assembly, the assembly materials need to have some affinity for each other, and therefore standard polyelectrolytes, such as dextran sulfate and polyarginine can be used, or hydrogen bonding materials such as tannic acid and poly(vinyl pyrrolidone) can be used instead.^{316,317} Importantly, antigens like ovalbumin, and bacterial adhesion molecules can be added to the mixture before spraying, thereby providing an easy means of high-efficiency drug loading.³¹⁸ Chemical crosslinking can be engineered to occur during the spraying process, which can allow for the formation of particles that shrink and swell under varied redox conditions.³¹⁹ One potential issue is that the

atomization process requires heat, although stable enzymes, such as horseradish peroxidase, can still be active after the formation process.³²⁰

Figure 40. (A) Schematic illustration of spray assembling particles with quasi-LbL assembly. (B) SEM, fluorescence microscopy and TEM images of the formed particles. Reproduced with permission from ref 31. Copyright 2014 Royal Society of Chemistry.

4.2 Other Constructs of Polyelectrolyte Complexes

As seen in the unconventional assemblies section of the review, PECs can act as a useful LbL assembly material. However, PECs can be utilized for much more than just LbL films, as demonstrated above in the quasi-LbL spray section. PECs can be used to form thin films at interfaces, or deposited in a single step, or even constructed into complex reformable plastics.³²¹⁻³²³ Although not truly LbL assembly, these methodologies and the materials they employ share numerous similarities with LbL, making them of interest for the thin film and plastics community.

4.2.1 PEC Films

An early innovation in the formation of PEC films is the use of sedimentation to assemble PECs onto a substrate. Stoichiometric PECs can be sedimented in a one-step approach with a significant fraction of the polyelectrolytes initially present in the solution deposited onto the substrate, however this technique relies on long sedimentation times, upwards of 24 h.³²¹ The size of the formed PECs, and consequently the sedimentation rate of the PECs, is dependent on the salt concentration. Moreover, this can result in two distinct film morphologies as in the simultaneous spray studies (section 4.1.1), where continuous homogeneous films are obtained upon sedimentation of PECs that display a high internal mobility (i.e., systems that show exponential film growth upon traditional LbL deposition). In this case, the film growth rate is directly proportional to the sedimentation rate of the PECs. On the other hand, a snowflake-like film morphology can be generated when the interactions leading to PEC formation are strong (i.e., systems that show linear film growth in conventional LbL deposition). Homogeneous film formation relies on the coalescence of deposited PECs and subsequent intermixing, whereas the absence of intermixing leads to a snowflake-like, more granular film morphology (**Figure 41**). This is similar to the results seen with unconventional assemblies of PECs,²³⁹ and also consistent with quasi-LbL spray assembly results.³¹⁵

Figure 41. Schematic illustration of quasi-LbL assembly using sedimentation of PECs, yielding either (A) a homogeneous film as the complexes undergo coalescence and intermix their contents when deposited onto the surface, or (B) a heterogeneous “snowflake” film where the complexes do not undergo coalescence after deposition. Reproduced with permission from ref 321. Copyright 2013 American Chemical Society.

Uniform coatings can be easily achieved by traditional LbL assembly, however films thicker than 1 μm are rather time-consuming to obtain because of the deposition of the individual layers and lengthy procedures generally used. Inspired by historical work on PECs,³²³ smooth films can be prepared by spin-coating stoichiometric (1:1) complexes of PSS and PDADMAC, when these two components are prepared as near-stoichiometric coacervates in the presence of sufficient potassium bromide (**Figure 42**).³²⁴ This spin-coating can lead to clear free-standing films of a thickness of $\sim 15\ \mu\text{m}$ in less than a minute. To form films of similar thicknesses via traditional immersive LbL assembly can take upwards of two weeks, and even spray LbL assembly would take approximately a day using similar conditions.

Figure 42. Schematic illustration of the spin coating process of transparent free-standing coacervate films. Reproduced with permission from ref 324. Copyright 2015 American Chemical Society.

4.2.2 PEC Plastics

For more than 50 years, dry PECs were considered to be unprocessable because of the observed infusibility and brittleness.³²⁵ It is well-established that salts can influence the formation and complexation of polyelectrolytes.³²³ PECs and coacervates fall into a continuum that has been studied extensively,³²⁶ where PECs (solid-like) are generally made without salt and PE coacervates (elastic liquid-like) generally contain salt ions. The difference between the two was further investigated using PSS and PDADMAC in aqueous solutions in the presence of different potassium bromide concentrations. Mixing equimolar amounts of PSS and PDADMAC resulted in a 1:1 ratio for PECs, however a significant divergence in ratio was observed for PE coacervates.³²⁶ This issue was recently overcome by making use of the salt-induced softening of PECs, which was known for decades, but previously unused in this application.³²² This salt-softening behavior is defined as “saloplasticity”, in analogy to thermoplasticity. PECs composed of PDADMAC and PSS or PMA can be compacted

in solutions of high ionic strength through centrifugation. The highly porous structure of the formed gels is attributed to excess PSS causing differences in osmotic pressure relative to solutions at lower ionic strength, and therefore the pore size can be easily controlled.³²⁷ Moreover, most pores can be reduced below 10 μm in diameter by extrusion.³²⁸ Tubes, tapes, and rods can be produced by extruding PECs plasticized with saltwater (**Figure 43**), with the presence of water critical for extrusion as even slightly dehydrated PECs are too brittle for processing.³²⁸ Saloplastics represent a novel approach to dealing with PECs that could have an impact on research and industry.

Figure 43. Images of (A) an extruded PEC film, (B) an extruded PEC rod, (C) an extruded PEC tube, and (D) its cross-section. Scale bars are 0.5 mm. Reproduced with permission from ref 328. Copyright 2012 Wiley.

4.2.3 PEC Capsules

Like LbL assembled thin films, PECs and coacervates can be used to form capsules for the encapsulation of organic and inorganic materials.³²⁹ For example, the coacervation of gelatin and gum arabic in the presence of acid³³⁰ can be used

to encapsulate colorless dyes, and also for preparing “carbonless” paper.³²⁹ However, PECs can also be used to prepare microcapsules including PEC-based LbL assembly²⁷³ and PEC-based encapsulation.³³¹ The latter approach has been used to prepare capsules by the drop-wise addition of cellulose sulfate to an aqueous solution of PDAMA, resulting in ~1-50 μm thick capsules.³³¹ Microdroplets can also be used to form PEC microcapsules using a microfluidic process based on the formation of PECs at the interface of a water/oil droplet (**Figure 44**).³³² Similarly, nanoparticle-polymer and protein-polymer composite microcapsules can be prepared with the same technique.³³³ More complex set ups, where the PECs are formed at the inner water/oil (W/O) interface of W/O/W double emulsions followed by spontaneous emulsion hatching, can also be used for microcapsule formation.³³⁴

Besides macro- and microdroplets, aerosol droplets can be used to form PEC capsules. In a surfactant- and template-free approach, PEC capsules can be prepared by spraying salt-doped PEC solutions. The capsule diameter and wall thickness depend on different parameters, including droplet size, temperature, polymer molecular weight and concentration, surface tension, and viscosity.³³⁵ A trend amongst quasi-LbL assembled PEC films is that the PEC films are generally orders of magnitude thicker than films prepared with the same polymers using LbL assembly.

Figure 44. Schematic illustration of quasi-LbL capsule assembly using microfluidics. (a) Aqueous solution of polyelectrolyte (blue) and an oil containing polyelectrolyte (yellow) are brought into contact to form microcapsules. (b) Electrostatic interaction of the polycation (red) and polyanion (blue) across the droplet interface for film formation. (c) Optical microscopy image of monodisperse stable capsules. Reproduced with permission from ref 332. Copyright 2014 Royal Society of Chemistry.

Spray-based capsule systems typically result in capsules in the tens of micrometers, which may be unsuitable for some biomedical applications. Approaches using solid particulate substrates impregnated with polymer can be used to make submicrometer PEC capsules.³³⁶ Monodisperse polymer-stabilized calcium carbonate (CaCO_3) particles can be formed by synthesizing CaCO_3 in the presence of anionic polymers (**Figure 45**).³³⁷ The size and shape of the particles can be controlled through the choice of stabilizing polymer (e.g., PSS, PGA,), reaction time, and feed ratios of calcium and carbonate. After formation, the particles can be loaded with drugs at a high efficiency, and only a single capping layer of polymer (e.g., PAH, PLL, chitosan) is needed to form the PEC capsules after core dissolution. Of note is that DOX itself can

be used to form acid-degradable particles with PSS using this technique. Such capsules have been used *in vivo* to demonstrate plaque targeting in a mice model.³³⁸

Another solution-based approach to form films around degradable substrates is through the use of metal-phenolic networks (MPNs).³³⁹ Small phenolic ligands such as gallic acid,³⁴⁰ or large polyphenols like TA,²⁰⁹ can spontaneously form films in the presence of metal ions. A wide variety of phenolic groups and metals can be used,^{341,342} and polymers can be functionalized with catechol moieties to allow for the spontaneous generation of hydrogels.^{343,344} MPN replica particles can also be formed this way through the use of porous particulate substrates.³⁴⁵ Finally, other linkers, such as boronic acid can be used instead of metal ions to spontaneously form MPN-like sugar-sensitive particles.³⁴⁶ Regardless of whether liquid or solid templates are used, PEC capsules can be formed rapidly, making these techniques applicable for a wide variety of applications.

Figure 45. Schematic illustration of the formation of PEC films using polymer-stabilized CaCO_3 particles. Adapted with permission from ref 337. Copyright 2015 Wiley.

4.2.4 Electrochemically-Assembled Polymer Films

The electrodeposition of polymers has a long history of use in industry for coating applications, such as the electrocoagulation of waterborne polymers,³⁴⁷ and can be exploited for continuously depositing films. A modest anodic potential can allow for the adsorption of certain amine side chain-containing polycations onto a conducting surface. This process leads to continuous linear film growth over time without the saturation usually observed for polyelectrolyte adsorption.³⁴⁸ This can be attributed to a suppression of electrostatic repulsion and/or ionic correlations arising close to a surface held at a constant potential. Therefore, polymer-polymer binding can be mediated by short-range interactions such as van der Waals or hydrogen bonding. “Click chemistry”³⁴⁹ can be used to generate covalently bound LbL assembled films composed of a single PE component,³⁵⁰ which can be extended to construct films in a step-wise manner by an electrochemically triggered Sharpless click reaction where Cu(I) is generated *in situ* from Cu(II) .¹⁹⁵ Moving away from conventional LbL assembly, this approach can be extended to a morphogen-driven one-pot polymeric film assembly method through the spatially confined Cu(I) -catalyzed electrochemical click reaction.³⁵¹ Specifically, two polymers separately bearing azide and alkyne groups can be crosslinked with Cu(I) ions, which were electrochemically generated continuously from a Cu(II) solution using a cycling potential (**Figure 46**). Similarly, continuous film growth from a single component polyelectrolyte using *in situ* generated polyampholytes in acidic pH can be engineered to occur simultaneously with self-complexation.³⁵² The required drop in pH is achieved through the spatially confined generation of protons by the oxidation

of hydroquinone under a constant current. Thermal crosslinking yields stable polyampholyte films that otherwise disassemble in the absence of current. Moreover, this use of acidic microenvironments allows for the formation of films from traditional LbL building blocks, like PAH/PSS, using the charge-shifting polyanion, dimethylmaleic-modified PAH, which is hydrolyzed into PAH under acidic conditions.³⁵³ This can be generalized even further, where PSS is replaced with an enzyme. Enzymatic activity is correlated with film thickness, however the activity eventually reaches a plateau, likely due to reduced enzyme accessibility from limited substrate diffusion into and through the film. Because of its spatial confinement, morphogen-driven film growth is promising for creating complex architectures on surfaces, especially when other sources for proton generation or other types of morphogens are implemented.

Figure 46. Quasi-LbL assembly using the one-pot morphogen-driven formation of films via electrochemically-controlled click chemistry. Reproduced with permission from ref 354. Copyright 2011 Wiley.

Major parts of what we consider quasi-LbL assembly are based on the formation of polyelectrolyte complexes (PECs) and PE coacervates. These have been extensively studied by Michaels,³²³ Kabanov,³⁵⁵ Tsuchida,³⁵⁶ and Dautzenberg.³³¹ The knowledge gained from these studies, in combination with

technologies developed in the field of LbL assembly, has driven the processability of PECs/coacervates, and the deposition of these materials into thin films far thicker than their LbL counterparts. These developments promote the application of a variety of different polyelectrolyte systems and has yielded a wider and more diverse toolbox to those studying and using thin films. Although quasi-LbL assembly is distinct from conventional LbL assembly, it marks a significant innovation in the thin film assembly field, as it represents an interesting alternative to repeated sequential assembly.

5 CHARACTERIZATION OF LbL FILMS

When assembling multilayer thin films, it is crucial to confirm that the layer materials are deposited as intended. After confirmation of film deposition, the growth process can be quantified and the resultant film properties, such as porosity, thickness, etc. can be determined. Characterization plays a crucial role in understanding assembly methods and the resulting films, and characterization techniques are therefore essential for guiding the development of new films and assembly methods. Characterization is also needed for differentiating between various assembly techniques, and allows for insights that can direct the films toward specific applications. For example, spin assembly can yield thick yet transparent multilayer films, which are otherwise difficult to prepare using immersive assembly and useful for applications in optics.¹²⁰ Generally, characterization methods for LbL assembled films are dependent on the substrate used, with thin films on planar substrates requiring different characterization methods compared with films on particulate substrates.

Further, some film properties are only easily observable using either planar or particulate substrates, while certain techniques require specific materials and film properties (**Figure 47**). As the techniques for characterization of LbL assembly and the resultant multilayer films have not been reviewed in this way previously, this section can also act as a guide on how to approach the study of LbL assembly and of LbL assembled films. References in this section are non-exhaustive examples that do not imply priority, but are intended to act as clear examples of how characterization methods can be used.

5.1 Characterization of Films on Planar Substrates

As seen in the above sections, planar substrates are relatively easy to handle and manipulate, making some forms of characterization straightforward due to the long history of studying macroscopic objects in other fields. For example, light-based techniques are a powerful tool for film characterization, as planar substrates can afford transparency, reflective properties, or surface-enhanced properties. Therefore, different techniques utilizing the whole electromagnetic spectrum ranging from X-rays to ultraviolet to infrared can be used to characterize films on planar substrates. Other techniques based on mass adsorption, forces, and electrons can also be used to provide additional information.

5.1.1 Assessing Film Growth

QCM is one of the main characterization methods for thin films.^{35,357} QCM devices can be used as is or fitted with microfluidics for the evaluation of LbL assembly *in situ* and *ex situ*, by measuring the changes in frequency and dissipation of a quartz crystal resonator typically coated with gold.^{358,359}

Initially, QCM was used to characterize protein (myoglobin or lysozyme) and polyion (PSS) assemblies.³⁵ In a series of important studies in the field, a number of researchers subsequently extended QCM by applying it to examine LbL-assembled films.^{35,358-361} Since then, QCM has become one of the most widely used and easily accessible methods for evaluation of the LbL assembly process and *in situ* film growth due to its small (benchtop) size, ease of use, and applicability to almost any layering material. In addition, QCM is capable of measuring sub-nanogram levels of materials, and can therefore be used to quantify the mass of deposited materials or the mass of the film itself based on the Sauerbrey equation:³⁶²

$$\Delta f = -\frac{2f_o^2}{A(\mu_q\rho_q)^{1/2}}\Delta m$$

where Δf is the measured frequency change (Hz), f_o is the resonance frequency (Hz), Δm is the mass change (g), A is the piezoelectrically active surface area of the quartz crystal (cm²), μ_q is the shear modulus of the quartz crystal (2.947×10^{11} g cm⁻¹ s⁻²), and ρ_q is the density of the quartz crystal (2.648 g cm⁻³). The QCM crystal frequency change typically includes the mass of water within the films, and so drying or the inclusion of appropriate controls or instrumentation can be required. Additionally, it should be noted that the equation is only valid when the deposited films are uniformly distributed, the magnitude of the film mass is less than the mass of the quartz crystal sensor, and the adsorbed films are rigid.³⁶³ Soft or flexible films can result in the dampening of the oscillation, however where the dissipation factor is generally above 10^{-6} per change of 10 Hz a Voigt viscoelastic model can be applied to characterize the films.³⁶⁴ Alternatively, in-house built QCM

devices with a frequency counter can be used to determine the mass of air-dried LbL films after each adsorption step.³⁶⁵

A Characterization of films on planar substrates

B Characterization of films on particulate substrates

Abbreviations:

AFM, atomic force microscopy; CLSM, confocal laser scanning microscopy; CV, cyclic voltammetry; DPI, dual polarization interferometry; DMA, dynamic mechanical analysis; DSC, differential scanning calorimetry; FT-IR, Fourier transform infrared spectroscopy; NMR, nuclear magnetic resonance; NR, neutron reflectometry; PAS, positron annihilation spectroscopy; QCM, quartz crystal microbalance; SAXS, small angle X-ray scattering; SERS, surface enhanced Raman spectroscopy; SPR, surface plasmon resonance spectroscopy; SEM, scanning electron microscopy; SIM, structured illumination microscopy; STORM, stochastic optical reconstruction microscopy; TEM, transmission electron microscopy; UV-Vis, ultraviolet-visible spectroscopy; XPS, X-ray photoelectron spectroscopy; XRD, X-ray diffraction; XRR, X-ray reflectometry.

Figure 47. Schematic illustrations of different characterization techniques applicable to characterize thin films on (A) planar and (B) particulate substrates. Different techniques can “cut across” substrate type and can be used for confirming or quantifying various film properties. This figure is intended to provide a general overview and is not exhaustive.

Other techniques for measuring the properties and kinetic analysis of LbL films *in situ* include ellipsometry, surface plasmon resonance (SPR) spectroscopy, and dual polarization interferometry (DPI). Ellipsometry is an optical method that detects the polarization changes of light upon reflection from a planar surface, hence the benefit of a planar substrate.^{43,366} This method can be used to measure the mean thickness (d_f) and refractive index (n_f) of films based on changes in ellipsometric angles (i.e., ψ and Δ).³⁶⁷ The mean thickness and refractive index can then be used to calculate the adsorbed polymer mass.³⁶⁸ The substrates for ellipsometry can be silicon wafers, glass slides, or metal substrates. SPR spectroscopy is also an optical method, but instead relies on the excitation of collective charge oscillations (i.e., surface plasmons) as they propagate on a planar metal surface (e.g., gold or silver) or onto the surface of metal nanoparticles. The resonance of excited surface plasmons is sensitive to the change in refractive index near the metal surface, and therefore SPR data is commonly recorded as reflectivity versus angle of incidence.³⁶⁹ SPR spectroscopy has been used to measure the kinetics of multilayer formation as well as the film thickness and refractive index,^{370,371} which has made it useful for conducting bioassays on LbL films.³⁷² SPR, like ellipsometry, can also be used to characterize air-dried LbL films, which is useful for studying the influence of pH and polymer molecular weight on film growth.³⁷³⁻³⁷⁶ However, the dry film thickness can decrease ~70% of that of the hydrated film, which has to be accounted for.³⁷⁷ DPI is another common optical characterization method and is based on the change in spatial positioning of two interference fringes emerging from reference and sensing waveguides. More specifically, DPI is

an evanescent field-based optical technique that uses the changes in spatial positioning of two interference patterns (birefringes) emerging from the reference and sensing waveguides to measure the thickness and refractive index of the deposited layer *in situ*.¹⁷ Detailed descriptions of DPI for film thickness characterization can be found elsewhere.^{378,379} This method has been used to characterize and quantify the film growth *in situ* for the LbL assembly of DNA,³⁸⁰ polyelectrolytes,³⁸¹ and liposomes.³⁸²

Film growth can also be evaluated with other parts of the light spectrum. For example, UV-Vis spectrophotometry can be used if the adsorbed materials (e.g., polymers, graphene, or nanoparticles) have specific absorption bands and the substrate is transparent.^{361,383-385} The absorption will increase relative to the amount of material deposited in each layer. This makes it easy to monitor film growth and determine whether the films grow exponentially or linearly. Fluorescence intensity and spectra can also be used to check the film growth when using fluorescent building blocks.³⁸⁶ However, the films generally need to be air-dried after adsorption of each layer. Instead of using the UV to visible range, X-rays can also be used to characterize film growth. For example, small angle X-ray scattering (SAXS) can be used to measure the total film thickness.⁷ XRD of stereocomplexed multilayer films can be used to demonstrate two distinct peaks characteristic to the PMMA stereocomplex before and after template etching.²³⁴ Also in the X-ray range, X-ray photoelectron spectroscopy (XPS) can be used to measure the surface atomic composition of films made of polymers or nanoparticles.^{373,387} In the IR range, Fourier transform infrared (FT-IR) spectroscopy not only allows for the monitoring of film growth but also allows

for the confirmation of chemical interactions (e.g., hydrogen bonding) between multilayers.³⁸⁸⁻³⁹⁰ Moreover, surface enhanced Raman spectroscopy (SERS) has been used to characterize LbL films incorporating metallic nanoparticles or carbon nanotubes.^{391,392} Many of the techniques applicable to monitoring film growth also give other relevant information.

5.1.2 Examining Film Morphology

The morphology of films can be analyzed by various microscopy techniques, including scanning electron microscopy (SEM) (**Figure 48**),³⁹³⁻³⁹⁵ transmission electron microscopy (TEM),^{396,397} confocal laser scanning microscopy (CLSM),³⁹⁸ and atomic force microscopy (AFM).³⁶⁰ Samples for SEM usually require a metal or carbon coating on the films to impart conductivity to the films, and therefore some metal sputtering techniques can result in nanostructure artifacts that may not be due to the LbL films themselves. TEM is usually used to identify nanoparticles in LbL films and to identify thick LbL films prepared or transferred onto copper grids.³⁹⁹ For CLSM, the materials of interest are typically labeled with fluorescent dyes, which can also be used to evaluate the polymer distribution in the LbL films.⁴⁰⁰ CLSM allows direct 3D visualization of the film construction, which facilitated the understanding of the diffusion-based buildup mechanism for exponential-like growth processes.⁴⁰⁰ In contrast, AFM is more versatile in characterizing surface morphology, topography, and root-mean-square roughness of LbL films, either air-dried or in solution.⁴⁰¹⁻⁴⁰³

Figure 48. Cross-section SEM images of $\text{Ca}_2\text{Nb}_3\text{O}_{10}$ films with (a) 3 layers, (b) 5 layers, and (c) 10 layers. Reproduced with permission from ref 395. Copyright 2010 American Chemical Society.

Film roughness can easily be monitored by water contact angle analysis, which has proven useful for studying electrochemically modified LbL films and also patterned LbL films (**Figure 49**).^{181,260} For example, approaching and receding contact angles of water on LbL films were used to demonstrate the independence of surface roughness on layer number for spin assembled films. In contrast, the contact angle strongly depends on the layer number for immersive assembled films.¹²⁰ Static contact angles on the other hand can help determine surface roughness.²⁶⁰ Neutron and X-Ray reflectometry²⁴² (NR and XRR) can also be used to determine film thickness, roughness and density, and have been used to compare immersive and spray assembled LbL films.⁴⁰⁴ For conductive films, cyclic voltammetry (CV) can be used to determine the electrical response and layer growth of multilayer films made from building blocks that exhibit redox potentials²⁶³ (e.g., carbon nanotubes,⁴⁰⁵ polymers,⁴⁰⁶ nanoparticles,⁴⁰⁷ and graphene.)⁴⁰⁸ Magnetization is less common, but can also be used to study the film packing, thickness, and morphology when using magnetic components for film growth.^{83,409,410}

Besides the methods mentioned above for characterizing film growth (i.e., DPI, SPR, and SAXS), SEM is an alternative approach to examine the film thickness by measuring the cross-section of films on broken silicon wafers or gold chips. This is usually performed to examine films that are >50 nm; for example, when the polymer layer number is well above 10 or when nanoparticles are used as building blocks.³⁵⁹ AFM has a high (sub-nanometer) resolution and is a common method for examining the thickness of films that have been “scratched” using a scalpel or an AFM tip.^{402,411,412} The AFM “scratch method” is applicable to thin films and it usually takes a long time to make a furrow; however, an advantage is that the sample does not need to be moved because the scratching and the measurements are performed with the same apparatus. The samples are typically prepared on glass or silicon wafers so that scratching does not damage the substrates and lead to artificial thicknesses.

Figure 49. Water contact angle analysis of patterned photoswitchable multilayer films. Reproduced with permission from ref 260. Copyright 2006 American Chemical Society.

5.1.3 Determining Internal Film Structure

LbL films are typically porous, with free volumes throughout the films, resulting from the polyelectrolytes as they complex, or from voids between nanoparticles. Using

nuclear magnetic resonance (NMR) cryoporometry a pore size of 1 nm has been reported within planar PAH/PSS films.⁴¹³ Alternatively, a number of neutral molecules or probe molecules can be used to determine the pore size of PAH/PSS films,^{414,415} and the results were consistent with the NMR cryoporometry data. However, the nano-sized porosity in LbL films has been relatively less investigated than other physiochemical properties. Instead of measuring specific nano-porosity, positron annihilation spectroscopy (PAS) has been used to investigate the concentration and size of free volume within thin films, which helps to predict the molecular and ionic transport properties.^{416,417} The effect of polymer composition, layer number, ionic strength, polymer molecular weight, and solution pH on the free volume element size and concentration within LbL films has been investigated using PAS.⁴¹⁸ Similarly, XRR and NR are common techniques for studying the internal structure of films and substrate effects on film structure.^{117,242,419,420} NR allows for detailed analysis of the internal film structure, including hydration states, and has helped elucidate the kinetics and driving forces of different LbL assembly techniques.¹³⁴ The conformation or orientation of the polymer chains in the film also influence the porosity, which has been examined using infrared spectroscopy,⁴²¹ as well as sum frequency generation vibrational spectroscopy and XPS.⁴²²

5.1.4 Assessing Mechanical and Thermal Properties

Dynamic mechanical analysis (DMA)^{423,424} and differential scanning calorimetry (DSC)⁴²⁵ can be used to examine the mechanical and thermal properties of LbL films. For example, stereocomplex formation was confirmed with DSC and it was

found that the melting point (T_m) of a stereocomplex multilayer film of PLLA and PDLA was 232 °C, while the T_m of single-polymer films of PLLA or PDLA were both ca. 170 °C.²³⁵ However, both DSC and DMA are limited to cases where a substantial amount of film material is present. In contrast, the stiffness and Young's modulus of thin films can be investigated using AFM,⁴²⁶ which is able to quantify mechanical properties for low Young's modulus films through small and controlled surface deformation.⁴²⁷⁻⁴²⁹ Polymer capsules are generally easier to study in terms of thermal properties, as changes can be viewed directly under an optical microscope.⁴³⁰

5.2 Characterization of Films on Particulate Substrates

Where planar substrates have advantages in terms of handling and processing, particulate substrates offer many unique advantages in terms of thin film characterization.⁴³¹ For example, particulate substrates easily allow for the charge reversal associated with LbL assembly to be measured.⁹⁴ Moreover, microscopy based techniques can allow for easy visualization of film thickness and permeability. Other techniques based on diffusion and light scattering can also be used with particulate substrates.

5.2.1 Assessing Film Growth

Microelectrophoresis can be used to determine ζ -potentials, and is one of the most commonly used methods for monitoring the LbL assembly process on particulate substrates, as the alternative deposition of polyelectrolytes on different templates (e.g., PS particles, oil droplets, and bubbles) often

causes a reversal in surface charge.^{72,94,98,432-434} Flow cytometry can be used to monitor polymer deposition on particles, where the scattered light or fluorescence (in the case of fluorescently labeled polymers) increases with layer number.^{374,435} Polymer films can change the scattering properties, and also the increased size can lead to reduced Brownian motion. Additionally, both flow cytometry and DLS can be used to determine particle aggregation during layering, as the scattering of doublets, triplets or larger aggregates have higher scattering than single particles.⁴³⁶ Besides DLS, LbL assembly on dense particles can be monitored using TEM after each layer and by using the contrast between particulate substrates and the polymer films to measure growth.⁴³⁶

5.2.2 Examining Film Morphology

The morphology and thickness of LbL films assembled on particles can be examined using AFM, SEM and TEM (**Figure 50**).^{39,437,438} A common way to study thin films is to remove the particulate substrate that the films have been deposited on, to yield hollow capsules.^{39,437,438} A convenient method to investigate that continuous LbL films have been deposited is to form capsules in aqueous solution and image them with optical microscopy, where non-continuous coatings just lead to fragments and debris after template removal. Differential interference contrast (DIC) microscopy can be used to image capsules (larger than around 500 nm) with high interference contrast.^{106,189} Similarly, fluorescence microscopy (e.g., CLSM) can be used to investigate capsules composed of fluorescent building blocks.⁴³⁹ Advanced super-resolution microscopy techniques⁴⁴⁰ (e.g., stochastic optical reconstruction microscopy (STORM) or structured

illumination microscopy (SIM)) are relatively new techniques that have recently been used to image small capsules (>100 nm) and determine the nanostructure of capsules.^{106,189} When liposomes are used as building blocks for the formation of capsosomes, cryo-TEM can be used to examine the presence of liposomes in the LbL capsules.⁴⁴¹ Cryo-TEM can also be used to assess capsules below 250 nm in diameter and cryo-electron tomography (3D cryo-TEM) can be applied to measure three dimensional nanoscale details of capsules.⁴⁴² Furthermore, immobilized particle imaging based on fluorescence microscopy can be used to characterize particles and capsules.⁴⁴³ Immobilization prevents the particles from moving and diffusing, and therefore a specific volume of sample can be imaged, which allows for exact concentrations of small particles to be determined.

Figure 50. a) SEM image of (PSS/PAH)₄/PSS capsules after removal of the MF core. The drying process induces folds and creases. b) TEM image of (PSS/PAH)₄/PSS polyelectrolyte shells. The template MF particles have a diameter of 2 μm. The stained multilayers surrounding the less stained interiors can be clearly identified and the layer thickness is of the order of 20 nm. Adapted with permission from ref 39. Copyright 1998 Wiley.

Air-drying LbL capsules typically results in collapsed capsules with creases and folds due to evaporation of the

solvent. Therefore, AFM is commonly applied to measure the double wall thickness of LbL capsules, which can be used to roughly calculate the single wall thickness and single layer thickness (**Figure 51**).^{39,209,444-446} Furthermore, AFM can be used to determine the root mean square roughness and other nanostructures of the film.⁴⁰² When nanoparticles are incorporated into the capsule shell, capsules can be freestanding even under vacuum, and ultramicrotomed slices and TEM can be used to measure the LbL film thickness.⁴⁴⁷ In addition, CLSM, AFM, and SEM have been used to examine the responsiveness of LbL films against external environment changes, such as temperature, pH, and solvent.^{52,448,449}

5.2.3 Determining Film Stiffness and Permeability

The mechanical properties of LbL capsules can be investigated by either studying the swelling of microcapsules filled with a solution of strong polyelectrolyte (e.g., PSS)⁴⁵⁰ or by measuring the deformation of LbL capsules under applied load using an AFM.⁴⁵¹ Specifically, the Young's modulus of many polymer LbL capsules in aqueous solution is in the range of 1-100 MPa.⁴⁵²⁻⁴⁵⁵ A combination of AFM and CLSM can be used to monitor the *in situ* deformation of capsules in the presence of applied force during mechanical measurements.^{454,456,457} Flow-based experiments in microfluidic chambers can be used to monitor the deformability of capsules.⁴⁵⁸⁻⁴⁶⁰ Fluorescence microscopy (e.g., CLSM) can be applied to examine the permeability of films in capsule form using fluorescent small molecules, polymers (e.g., dextran), or nanoparticles under different solution conditions.^{170,461-463} Permeability and stiffness are relatively

straightforward to measure, as the related characterization techniques have been used widely in related fields.

Figure 51. (a) Zeta-potential as a function of the number of layers. Layer 0 represents the bare silica particles (2.39 μm in diameter) before layering. Odd layer numbers are PDADMAC, and even layer numbers are PSS. (b) Fluorescence microscopy image of SiO₂ particles coated with (PAH-FITC/PSS)₄ multilayers in solution. (c, d) AFM image and corresponding cross section of air-dried (PDADMAC/PSS)₄ capsules. The position of the height profile in (d) is indicated with a white line in (c). Dotted line at 25 nm in (d) indicates approximate double wall thickness. Reproduced with permission from ref 171. Copyright 2015 American Chemical Society.

6 CONCLUSION AND OUTLOOK

LbL assembly has a long and rich history of technological integration and advancement, and a wide variety of methodologies have been established in the literature. The frontiers of LbL assembly are ever changing, and techniques and assemblies that have been relatively uncommon in the LbL field, will continue to be integrated into the everyday toolbox of the field. Although “quasi-LbL” assembly is fundamentally different than LbL assembly, it too has an important place in the field of LbL assembly, and can offer numerous insights into the properties of thin films and polyelectrolyte materials. Importantly, the characterization techniques for LbL assembly are well-established and allow for conventional, unconventional, and “quasi” assemblies to be studied and compared. Although this review is only a snapshot of the field as it currently stands, it is aimed towards aiding readers in understanding the thin films assembly techniques relevant to LbL assembly, and the experimental methods for characterizing those same films, to inspire future developments.

The future of the LbL assembly field is just as bright as its past. LbL assemblies will continue to move into diverse fields, and those same fields will contribute new ideas, methodologies, and characterization techniques back to LbL assembly. This is a trend that will only be further accelerated by the ongoing convergence of the physical sciences, engineering, and biomedicine.⁴⁶⁴ We envision that multilayers will become even more versatile and tailored as additive manufacturing methods continue to be integrated with LbL assembly. There are still numerous fabrication techniques that have not been integrated with LbL assembly, but only time

will tell if they can result in unique multilayer properties, or expedited deposition processes. Finally, there is still significant work to be undertaken in characterizing the various assembly processes in situ, and establishing methods and models for predicting the properties and performance of films based on the materials, conditions, and assembly methods. Computational approaches should play an important role in that regard. Developments and innovation in LbL assembly is happening faster than ever, and as the field continues to grow and evolve, it is likely that new branches will be added to expand past conventional, unconventional, and quasi LbL assembly.

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