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Layer-by-Layer Assembled Capsules and Films for Therapeutic Delivery

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Abstract

Polymeric materials formed via layer-by-layer (LbL) assembly have promise for use as drug delivery vehicles. These multilayered materials, both as capsules and thin films, can encapsulate a high payload of toxic or sensitive drugs, and can be readily engineered and functionalized with specific properties. This review highlights important and recent studies that advance the use of LbL assembled materials as therapeutic devices. It also seeks to

identify areas that require additional investigation for future development of the field. A variety of drug-loading methods and delivery routes are discussed. We also identify the biological barriers to successful delivery and discuss current solutions to these problems. Finally, we cover state-of-the-art degradation and cargo release mechanisms.

1. Introduction

Polymeric materials can be used to unlock the potential and improve the effectiveness of therapeutics. For example, encapsulation in a polymeric matrix can allow for controlled release of the drug and an improved therapeutic profile. Additionally, polymers can protect the body from the side effects of toxic chemotherapy agents in healthy tissue, or they can protect sensitive therapeutics like nucleic acids or proteins from degradation enzymes (nucleases or proteases) present in the body. Polymers are an enormous class of compounds that can be assembled into useful materials via a variety of methods. Polymer-drug conjugates,[1] self-assembled micelles,[2] particles formed via emulsion polymerization,[3] and dendrimers[4] are just a few of the different materials used in this area. A simple yet highly versatile process for assembling polymeric materials used as advanced drug delivery systems are multilayered films formed via Layer-by-layer (LbL) assembly.

The alternate deposition of oppositely charged particles to create a film of multiple layers was first described by Iler in 1966.[5] This concept was adapted 25 years later by Decher and colleagues for the formation of thin films using polymers of opposite charge.[6-8] LbL is a highly versatile process because it can be performed on planar or colloidal substrates of almost any surface chemistry with a variety of polymers and other components including (but not limited to) nanoparticles,[9] proteins,[10] DNA,[11] and viruses.[12] In the simplest example of LbL assembly, a charged substrate (e.g., negative) is immersed into a solution containing an oppositely charged polymer (**Figure 1a**). When the adsorption of the positively charged polymer reaches equilibrium, the substrate is rinsed and immersed into a negatively charged

polyelectrolyte solution. Adsorption of the polymer is irreversible at the time scale and conditions used and charge overcompensation results in charge reversal at the surface.[13] Because of these factors, the process can be repeated to form multilayered films.

LbL can also be performed on colloidal substrates (**Figure 1b**). The adsorption of the polymer onto the colloidal substrates occurs in the same way as on planar surfaces; however, to remove the polymer solution a cycle of centrifugation and resuspension steps is employed. The thickness can be controlled by altering the number of layers deposited during LbL assembly. When the desired thickness is obtained, the particles can be used in the core-shell state, or the core can be dissolved to leave hollow polymeric capsules. This review focuses chiefly on colloidal materials, although planar surfaces are considered for their application in the surface modification of macroscopic objects (e.g., for biomedical implant devices).

The use of LbL assembly has a number of advantages. For example, LbL assembly can be performed in entirely aqueous solutions, requiring no exposure to organic solvents. This is important for biomolecules such as nucleic acids and proteins, which have limited solubility in non-aqueous solutions and are susceptible to denaturation. The size and shape of the capsules can be engineered by simply altering the template used for polymer adsorption. A large range of polymers can be used to create the capsule wall, resulting in the ability to finely tune the composition, permeability, stability, and surface functionality of the capsules. The assembly process is relatively cheap, requiring only simple laboratory equipment, and can be performed with inexpensive materials. For these reasons, LbL assembly is considered a promising method for the creation of inexpensive, non-toxic, and efficient therapeutic delivery devices.[14]

There are specific requirements for the composition of the drug delivery devices. The therapeutic of interest should be appropriately incorporated into the delivery vehicle, i.e., it should shield toxic and fragile drugs from the body. The delivery vehicle should be designed for delivery at two levels: firstly it must evade the immune system and reach the correct tissue

or organ; then it must overcome internal cellular barriers so it reaches its site of action. All components must eventually be approved by the Food and Drug Administration (FDA) for use in humans. Thus, the polymers must have minimal toxicity, and be efficiently removed from the body without accumulation. In addition, the device must contain a mechanism for release of the encapsulated drugs. Above all, the material should be responsive to its surroundings, a topic of increasing interest in polymer science.[15] A recent review by Akashi discusses the use of multilayered capsules in targeted- and drug-delivery, focusing on their synthesis, loading and release.[16] Here we also address the factors affecting the route of delivery, internalization and toxicity, contrasting examples of current state-of-art research with LbL assembled materials.

2. Cargo Loading

The loading of cargo into LbL films and capsules can be broadly described by three methods: a) incorporation into the film; b) pre-loading the template used for LbL assembly; and c) post-loading by altering the permeability of the thin film and trapping the cargo inside **(Figure 2)**. The first and most widely used method is to incorporate the molecule of interest as one or more layers of the multilayered films (Figure 2a). This can be achieved by incorporating a layer of unmodified cargo (e.g., for natural polyelectrolytes, such as DNA, proteins, and sugars), or by conjugating the cargo to the polymer pre- or post- film assembly (e.g., for small molecules such as peptides or doxorubicin). Incorporation into the film is popular because it is suitable for use on planar surfaces such as macroscopic implantable devices, and also on particles for systemic delivery. There are several advantages to this method. LbL assembly of the cargo results in a uniformly loaded thin film, and the quantity of cargo loaded can be varied by increasing or decreasing the number of layers deposited. This is important for the delivery of an accurate and consistent dose of the therapeutic. The layered nature of the thin films facilitates the inclusion of multiple drugs within different layers of the same film, and

allows for their temporally controlled release. A disadvantage of incorporating the cargo into the film is that LbL is typically performed with an excess of polymer that is discarded once adsorption has reached equilibrium, resulting in cargo wastage. Additionally, the cargo is encapsulated in a complexed form, which can affect the properties of the cargo (e.g., activity or intracellular processing).

The second method for cargo loading involves pre-loading the template particle with the cargo prior to LbL assembly (Figure 2b). This results in the cargo being surrounded by a protective thin film and is highly suited to colloidal systems. The template may be a crystal, aggregate, or otherwise particulate form of the cargo, forming a core-shell particle after LbL assembly. This has been performed for crystallized small molecules,[17,18] condensed DNA,[19] and proteins in a crystallized[17] and amorphous[20] state. The size and shape of the final delivery vehicle is dependent on the type and quality of the template formed. Thus, monodisperse templates may be difficult to achieve. Alternatively, instead of the template being made from the cargo, it may be a sacrificial particle (often porous) into which the cargo is loaded. After LbL assembly, the template is dissolved away to leave a capsule with the cargo inside. This technique has been used for the encapsulation of DNA[21,22] and proteins.[23-27] Sacrificial templates are most commonly formed from silica or calcium carbonate. Silica particles have the advantage of a high degree of control over the porosity and size; however, they require the use of hydrofluoric acid (HF) to remove the core. Although harmful to living organisms, the use of buffered HF to dissolve silica template particles has been shown not to affect the function of DNA[21] or certain proteins.[27] Calcium carbonate can be dissolved in the very mild conditions of ethylenediaminetetraacetic acid; however, it offers less control over particle size and porosity. A combination of the two pre-loading methods was the addition of a protein to calcium carbonate template reagents during synthesis.[28,29] Pre-loading templates with cargo provides high encapsulation efficiency, particularly when the templates are made of the cargo.

The final method for cargo loading is to post-load an already formed capsule by allowing diffusion of the cargo inside the capsule and trapping it there (Figure 2c). This can be achieved by changing the permeability of the capsule wall to an “open” state and then returning it to a “closed” state in response to pH,[30] ionic strength,[31] or temperature. Alternatively, a sequestration reagent may be used to encourage diffusion into the capsule and to retain the cargo inside.[32] The encapsulation efficiency of post-loading is generally low and without the use of a sequestration reagent, it is difficult to significantly increase the concentration of cargo inside the capsule.

2.1. Small Molecules

The delivery of encapsulated small molecules confers several advantages over delivery of the free molecules themselves. For toxic drugs, such as chemotherapy agents, encapsulation can prevent access of the drug to healthy cells and minimize side effects. Additionally, encapsulation can allow the delivery of non-water soluble drugs that have limited efficiency when delivered by conventional means. Other drugs may benefit from localized or prolonged release that can improve the therapeutic profile. Because of the semi-permeable nature of LbL films, small molecules must be linked or complexed to prevent them from freely diffusing through the capsule wall.

Doxorubicin (DOX) is an anticancer drug commonly used in the clinic to treat many different types of cancer. One of its most serious side effects is toxicity to the heart.[33,34] DOX has been encapsulated within liposomes to reduce side effects on healthy tissue. Liposome-encapsulated DOX systems are some of the few nanotechnology-based healthcare products on the market. However, these first-generation products also cause toxic side effects, such as Hand-Foot Syndrome.[35,36] Alternative carriers are required to further reduce the toxicity of DOX in healthy tissue.

A novel method for the post-loading of hydrophobic molecules, such as DOX, is to encapsulate them in emulsions.[37] DOX and 5-fluorouracil (5-FU) were dissolved in oleic acid, which was then infiltrated into disulfide crosslinked poly(methacrylic acid) (PMA) capsules (**Figure 3**). The DOX was released when the capsules were placed in a reducing environment, such as the interior of a cell. Encapsulated delivery of the DOX decreased the half maximal inhibitory concentration (IC50) compared to the free drug in solution by seven orders of magnitude.[37] Thus, the administered dose can be lowered to achieve the same effect, resulting in decreased side effects. The same method used for loading oil into the capsules was used for liquid crystals.[37-39] The liquid crystal-filled capsules were used as biosensors for identifying whether bacteria are gram positive or negative, and whether or not viruses are enveloped.[40]

In another strategy, DOX was conjugated to a polymer and incorporated into a multilayered film on gold nanoparticles.[41] The DOX was linked to the polymer via an oligopeptide spacer that was a substrate for lysosomal enzymes. The DOX was released over 24 h in the presence of the enzymes. Other small molecules can benefit from encapsulation. The hydrophobic drugs, paclitaxel or diclofenac, were loaded into 90 nm copolymer micelles using an emulsion encapsulation method.[42] These drug-loaded micelles were then incorporated into a multilayered film containing a hydrolytically degradable poly(β-amino ester) and a model macromolecular drug, heparin sulfate. Release of the drugs was observed in vitro in cultured cells.

2.2 DNA

Despite the promising and extensive research into nucleic acid based therapeutics, such as gene therapy and small interfering RNA (siRNA), very few products are in use. This is because of the difficulty associated with delivering an intact nucleic acid drug to the action site in vivo. There are several barriers which must be overcome before the therapeutic reaches its target. In most cases it is not feasible to inject or ingest naked nucleic acids because they are quickly degraded by nucleases present in the blood stream, saliva etc. Two of the nucleic acid therapeutics currently on the market, Vitravene® and Macugen®, are both injected directly into their action sites in the eye to avoid degradation during transport. The other product, Gendicine®, which is approved for use only in China, is encapsulated within a viral carrier that provides a physical barrier against nucleases. An additional barrier to nucleic acid delivery is the lipid bilayer surrounding the cell. Nucleic acids are large and highly charged, and therefore have difficulty crossing the hydrophobic region of the membrane. Unaided uptake is inefficient and occurs through the process of endocytosis,[43] which may result in the DNA being trapped in an endosome or lysosome vesicle. Internalization of delivery vehicles by endocytosis can also result in fusion with lysosomes.[44] Thus, the vehicle should avoid the lysosome or incorporate a mechanism for escape. A robust and non-toxic carrier is required to protect the nucleic acid therapeutics from degradation, and improve their transport across the lipid bilayer.

LbL films and capsules can provide protection from nucleases by either condensing the DNA with a polycation or by using the thin film as a physical barrier. Additionally, the polymers can enhance cellular internalization. DNA is a strong polyelectrolyte that is negatively charged at almost any pH and can be electrostatically incorporated unmodified into the films by alternation with polycations. DNA was first incorporated into a planar LbL film with the positively charged synthetic polymer poly(allylamine hydrochloride) (PAH),[11] and has since been incorporated into increasingly sophisticated films. For example, DNA has been

alternated with polycationic polymers that are hydrolytically,[45] enzymatically[46] and reductively degradable.[47] This approach has been extensively used on planar supports and has allowed the controlled release of several plasmids (pDNA) embedded in different layers.[48,49] Recently, hydrolytically degradable films were extended from planar surfaces to colloidal surfaces: they were formed on the surface of 6 μm diameter poly(styrene) particles for DNA vaccine delivery.[50] These delivery vehicles were core-shell particles with 2.5-bilayers of poly(β-amino ester)/pDNA synthesized by LbL. Aggregation during LbL was a significant problem that was addressed by passing the particle suspension through a fine-gauge needle prior to polymer adsorption. The pDNA was functional in a cell after delivery using a standard transfection reagent.

DNA may also be encapsulated within the central void of hollow capsules using the template pre-loading technique (**Figure 4**). First the DNA is adsorbed onto the template particle, followed by multilayered film formation. After core removal, the DNA is entrapped inside the central void of the capsules. This was described for oligonucleotides[22] and plasmids.[21] The encapsulated DNA is free and uncomplexed, which allowed the capsules to be used as microreactors.[51]

Compared to DNA, there are few reports that detail the encapsulation of siRNA in LbL assembled systems. siRNA has been incorporated as a single layer in between two poly(ethyleneimine) (PEI) layers on gold nanoparticles,[52] and was incorporated as a pre-formed PEI/siRNA complex in a planar hyaluronic acid/chitosan film. PEI is a well-known transfection reagent,[53] and was able to promote the transfection of cells with the siRNA. The multilayered films could transfect cells with siRNA resulting in a reduction in specific protein production from both gold nanoparticles[52] and macroscopic planar surfaces.[54]

2.3 Proteins

Proteins have several roles in nanomedicine; they may be used as the therapeutic cargo (particularly enzymes) or as a structural component of the carrier vehicle. As a structural component, proteins are biodegradable, biocompatible and can have intrinsic targeting or complementary activity. For these reasons, drug delivery vehicles made from proteins have already found success in nanomedicine. For example, human serum albumin (HSA) is used as a carrier particle for paclitaxel in the nanotechnology-based chemotherapy agent Abraxane®. HSA improves the solubility of the drug without the use of solvents and improves its pharmacokinetic profile.[55] Proteins are easily incorporated into LbL assembled materials because they are natural polyelectrolytes.[56,57] Proteins are amphoteric and change their charge with pH. Hemoglobin has an isoelectric point of 6.8, and so was used as the cationic component of an electrostatically assembled film at pH 4.5 and as the negative component of an electrostatically assembled film at pH 9.2.[56] HSA nanotubes with potential as drug carriers were formed by LbL assembly with PAH or poly(L-lysine) (PLL) in a porous membrane that was subsequently removed, leaving cylindrical protein nanotubes.[58] In addition to their use as carriers, proteins may be used as therapeutic agents, for targeted delivery and as catalysts.

Encapsulated enzymes have great promise for catalysis. Immobilization or encapsulation of enzymes allows for their simple recovery from the products and protection from proteases. The first example of the use of multilayered films for enzymatic catalysis was performed using glucose oxidase and peroxidase, enzymes with linked reactions, on planar surfaces[59] and on colloids.[60] For use in therapeutic delivery, encapsulated enzymes may be used for the conversion of prodrugs into an active form, such as the anticancer prodrug Miproxifene, which is activated by alkaline phosphatases.[61] Enzymes were encapsulated within liposomes which were subsequently assembled into multilayered films on particles.[62] The core was dissolved away to produce capsosomes, polymer capsules with thousands of

subcompartments. The liposomes acted as protective compartments that separated the enzyme from its substrate. On the addition of a surfactant, the liposomes burst and the enzymes had access to the substrate. In the future, capsosomes may be used as artificial cells.[63] In another example, the activity of an encapsulated enzyme is reversibly switched on as a result of mechanical deformation of a multilayered film.[64] The enzymes were immobilized in a multilayered film on silicone substrates by alternation with an oppositely charged polyelectrolyte. Another capping film was assembled on top that prevented access to the enzymatic substrate. When the film was stretched by 70%, the top layer of enzyme was able to access the substrate and the reaction could proceed. However, when the film was returned to its original state, the enzyme was unproductive again. An enzymatic switch such as this may find use in mechanically controlled biopatches.

A multilayer formed from hemoglobin and glucose oxidase crosslinked via gluteraldehyde was found to increase in permeability when glucose was added to the system.[65] The enzymes have interlinked reactions: glucose oxidase catalyses the hydrolysis and oxidation of glucose to form gluconic acid and hydrogen peroxide, which is then reduced by hemoglobin. It was proposed that the reduction of pH resulting from gluconic acid caused the film to increase in permeability.[65] This has applications as a glucose microsensor, but is also interesting for triggered insulin release.[66] This was demonstrated using multilayers of glucose oxidase and catalase formed around insulin particles. When glucose was added, the production of gluconic acid and subsequent lowering of pH caused an increase in solubility of insulin.[66]

3. Delivery Routes

There are a number of routes that can be used to introduce the therapeutic to its active site in the body. The route of administration differs by drug and disease. For example, skin disease is often treated topically, whereas heart disease may be treated orally, by injection, or by implantation. Lipid soluble drugs can be administered orally as they are able to cross the

gastrointestinal tract into the blood stream, whereas macromolecular drugs such as vaccines should be administered by injection so they can bypass the harsh conditions of the gastrointestinal tract. The biodistribution of the therapeutic may depend on the route of administration,[67] which can alter the effectiveness of the drug. The delivery route may be directed by targeting the delivery vehicle to a specific cell or tissue type using surface functionalized “targeting” moieties. So far, LbL assembled materials have been developed chiefly for either implantation or injection. However, as materials scientists collaborate more with biologists and clinicians, new applications for LbL assembled materials will be found. Discussed here are the commonly considered administration routes for drug delivery from LbL assembled films.

3.1. Topical

In topical delivery the drug is applied externally and locally, and it then diffuses through the skin to the site of action. This may be achieved via a cream or a patch on the skin, such as a nicotine patch or wound dressing. Wound dressings have been mentioned as potential applications for LbL assembled films although no examples have been published thus far. However, in a similar application, LbL films have been used to coat surgical sutures (for use in stitching up wounds).[68] A multilayered film of PAH/dextran/hyaluronic acid loaded with ibuprofen was formed on silk surgical sutures with a diameter of 0.3 mm. The ibuprofen was released over several days in a saline solution. This therapeutic coating can be used to improve the healing and comfort of the patient. A hydrolytically degradable multilayered film containing a vaccine antigen was developed for the delivery of the drug across barrier-disrupted skin.[69] Ovalbumin was used as the model vaccine antigen and the film was formed on the surface of a flexible Poly(dimethylsiloxane) substrate (**Figure 5**). The film could be dried and stored, and then rapidly rehydrated prior to use. When applied as skin patches, these films could release ovalbumin and an adjuvant, cytosine-phosphate-guanosine (CpG)

oligonucleotides, to cells in the skin.[69] This system is of particular importance for non-refrigerated storage and needle-free application of vaccines.

3.2. Parenteral

Drugs with a parenteral route of administration are introduced into the body via injection or implantation. Although these techniques are invasive, they circumvent the epithelial barriers of the skin and gastrointestinal tract. Thus, drugs with properties unsuited to transdermal delivery or the environment of the gastrointestinal tract can still be effective via a parenteral route. Discussed here are recent examples of LbL assembled materials administered via injection or implantation.

3.2.1. Implantation

LbL is a useful technique for altering the surface of implants. It can be used to improve the biomaterial interface or for the controlled release of drugs. Controlled or prolonged release of therapeutics from implants is useful for a wide range of drugs that require constant or localized administration. A multilayered film containing a lipid antagonist was created for the treatment of bacterial infections by preventing the initiation of the septic shock pathway.[70] The lipid was complexed within the polypeptide film with a cyclodextran and was released in the presence of macrophage cells, resulting in the inhibition of cytokine production (compounds involved in septic shock). These films were proposed for use in surgical situations, but have only been investigated in vitro. In other surgical situations, the therapeutic effect of stents (devices that are implanted in the arteries or veins to counteract flow restrictions) can be improved by coating the stent with a drug reservoir with the capability of slow release.[71] An LbL assembled thin film was deposited on the surface of a stent.[72] The film was designed to slowly release DNA via hydrolytic degradation of the polymer to provide localized gene delivery of a model protein. It was uniformly deposited on the surface

of the stent and was not significantly disrupted when the stent was expanded or passed through a silicone septum and the shaft of an arterial inducer.[72]

Titanium is used in vivo for replacement of hard tissue in load-bearing applications such as dental implants and hip replacements, but is limited to passive integration with the surrounding soft tissue in vivo. Thin films can be used to improve the interaction of the titanium implant with the surrounding tissue. Several multilayered films have improved the interaction of titanium surface with cells: PLL/poly(L-glutamic acid),[73] gelatin/chitosan,[74] PLL/pDNA,[75] hyaluronic acid/chitosan/RGD,[76] and chitosan/pDNA[77]. An in vivo study has demonstrated that these films can indeed improve the biocompatibility of implants, and also that local release of drugs is possible.[73]

3.2.2. Injection

The vast majority of polymeric colloidal delivery vehicles are intended to be introduced into the body via injection. It is a comparatively direct method for accessing the internal organs that avoids the barriers of the skin and gastrointestinal tract. If injected delivery vehicles are required to travel from the injection site through the blood stream, they should fit within the blood vessels. Small capillaries have an average diameter of 7 μm,[78] and although the optimal size of delivery vehicles depends on the specific requirements of the drug or disease, this is a useful guide. There are several locations that the delivery vehicles can be injected, and each of these has a unique biodistribution. For example, polymeric nanoparticles injected intravenously, interperitoneally, or subcutaneously have different biodistributions in mice.[67] The nanoparticles that underwent interperitoneal injection showed increased uptake in the stomach and intestine compared to the other injection sites. To our knowledge, there are no published investigations of the in vivo biodistribution of LbL assembled capsules or core-shell particles. Ascertaining and optimizing the biodistribution is important for validating this technology for use as drug delivery vehicles. Optimizing the biodistribution will require

additional surface functionalization and particle engineering, and ideally should be performed simultaneously with engineering the particles for loading, degradation, and release. Although this area has been lacking thus far, it is likely to be extensively investigated in the future. One example of the use of capsules in vivo is the injection of disulfide crosslinked PMA capsules containing vaccine antigens into the tail vein of mice.[79] The spleen was harvested for analysis of specific T cell activation, and showed that the capsules were able to effectively deliver the protein/peptide cargo in vivo.

One of the advantages of encapsulated delivery is the possibility of targeting the delivery vehicles to a specific location or cell type within the body. Targeted delivery can increase the effectiveness of the drug while minimizing side effects. It can decrease the side effects of the therapy by restricting access of the drug to tissues in which it is not required. This is particularly important for toxic drugs like anticancer agents that are toxic in healthy tissue. Additionally, targeted delivery increases the local concentration of the therapeutic, requiring a lower dose to achieve the same efficiency. Targeted delivery can be achieved via several mechanisms: 1) passive targeting (including the enhanced permeability and retention (EPR) effect); and 2) surface functionalization of the delivery vehicle with a targeting ligand (an antibody, sugar, peptide etc.). The EPR effect is the phenomena where macromolecules and particles preferentially accumulate in tumors rather than in healthy tissue. This effect is exploited in current nanomedicines, although even these cause side effects in healthy tissues,[35,36] as discussed in Section 2. Surface functionalization with targeting moieties, such as antibodies, sugars or aptamers, allows the carrier to be directed to a specific organ or tissue type. It may also improve cellular uptake of the delivery vehicle by promoting receptor-mediated endocytosis. FDA-approved products with active targeting moieties are first-generation antibody-drug conjugates or fusion proteins, whereas next-generation nanoparticle carriers such as liposomes are in clinical trials.[80]

An example of polyelectrolyte capsules en route to targeted delivery is the specific delivery of functionalized capsules to colon cancer cells expressing an antigen.[81,82] Core-shell particles and capsules coated with a multilayered film were surface functionalized with an antibody against A33, an antigen expressed on colon cancer cells. The functionalized capsules showed a 4-fold increase in cell binding than capsules without the antibody. Capsules of 500 nm had lower non-specific binding to cells than 1 μm capsules.[82]

4. Cellular Internalization

After the delivery vehicle has been administered and is localized at the organ/tissue level, there are additional barriers at the cellular level before its therapeutic cargo can be effective. The first barrier to be overcome at the cellular level is the lipid bilayer membrane (**Figure 6**). The ability of molecules to directly cross the cellular membrane decreases with increasing size and charge. Without specific pores or pumps to cross the lipid bilayer, macromolecules (larger than 1 kDa) and particles rely on endocytosis for internalization. Endocytosis is the process whereby cells absorb material from outside the cell by engulfing it within their cell membrane. This results in the internalized material being contained within a lipid bilayer (known as an endosome – see Section 4.3). Endocytosis is a complex mechanism and can occur via a number of mechanisms.[83] Phagocytosis is one of these mechanisms, and is triggered in the presence of particulate material (such as synthetic particles, bacteria or viruses) and involves localized actin remodeling around the phagocytic cup on the surface of the cell. This process is primarily reserved for specialist phagocytic cells such as macrophages, neutrophils and dendritic cells, although it has been observed in other cell types.[84] Large particulate material can also be internalized by macropinocytosis. In contrast to phagocytosis, macropinocytosis can occur independently of the presence of a particle and is a common feature of almost all mammalian cells. The process involves the uptake of large amounts of fluid and so can result in the non-specific internalization of material from the

extra-cellular environment. The mechanism of macropinocytosis is also actin dependent. Other endocytic pathways, such as clathrin-mediated, caveolae-mediated and clathrin- and caveolae-independent mechanisms, are also involved in the internalization of material.[44] An investigation into the mechanism of uptake of 3 μm dextran sulfate/poly-L-arginine capsules proposed a macropinocytotic uptake route into African green monkey kidney cells.[85] More investigations are required to understand how LbL assembled materials are internalized inside cells.

4.1. Size

The size of the delivery vehicle being internalized can directly influence the mechanism for internalization. For example, clathrin-mediated endocytosis typically results in vesicles of 100 nm and caveolin-mediated endocytosis begins with membrane invaginations of 50-80 nm.[44] Thus, particles that are much larger than 100 nm are unlikely to be internalized by these endocytosis mechanisms (although there are documented exceptions of particles up to 500 nm that were internalized by caveolin-mediated endocytosis).[86] Large particles that cannot be internalized via these pathways are presumably internalized by phagocytosis or macropinocytosis, which can form macropinosomes as large as 10 μm. Although early studies showed that particles must be less than 500 nm to undergo cellular internalization by phagocytosis,[84] more recent investigations have shown that the internalization of much larger particles is possible.[87] For example, LbL assembled core-shell delivery vehicles of 1 μm,[81] 3 μm,[88] and even 6 μm[50] diameter have all been observed inside cells. The optimum size for cellular internalization is dependent on the cell line used, the surface chemistry, and the application. In the case of targeted delivery vehicles, the size should be optimized for increasing specific internalization over non-specific internalization. For example, although 1 μm particles showed higher binding to the surface of cells, 500 nm particles had improved specific binding.[82] Additionally, the size can be used to target specific types of cells or

internalization processes.[86] Lynn and colleagues have used the size of the particle to target the delivery vehicles to the type of cells required for DNA vaccination.[50] They used large 6 μm diameter particles because particles of that size are preferentially internalized by antigen-presenting cells over other cell types.[50] It is clear that there is no single size that will be suitable in all cases; but rather the size should be optimized for each application.

4.2 Shape and Elasticity

Recent studies have indicated that the shape of particles affects the rate and amount to which they are internalized by cells.[89] Some geometries, such as UFO-shaped discs[89] and high aspect ratio particles,[90] were able to prevent internalization by professional phagocytes alveolar macrophages, although this was also dependent on the geometry at the initial point of contact. The effect of aspect ratio appears to have the greatest effect when one dimension is greater than 1 μm. Below 100 nm the role of shape is not clear. It has been recently shown that there is no significant difference in the internalization of gold nanoparticles (15 nm) and gold nanorods (15 x 50 nm).[91] However, other studies with gold nanorods have shown rods with a high aspect ratio (14 x 40 nm and 14 x 74 nm) showed significantly lower uptake than spherical gold nanoparticles (both 14 nm and 74 nm).[92] Variations in the mechanical properties of delivery vehicles can also impact cellular internalization.[93] Soft hydrogel nanoparticles were internalized via macropinocytosis,[93] the same process determined for polyelectrolyte capsules;[85,94] whereas stiff hydrogel nanoparticles were internalized via a clathrin dependent process.[93] Non-degradable, hollow polyelectrolyte capsules were found to deform on internalization inside cells, although a decrease in deformation was noted when the capsules had been heat treated and shrunk prior to the investigation.[95] The capsules were formed from poly(styrene sulfonate) (PSS) and poly(diallyldimethylammonium chloride), and also contained gold nanoparticles embedded in the polymer shell. The presence of gold particles also decreased the deformation of the capsules after internalization. These

investigations highlight the importance of understanding the relationship between the mechanical properties of the capsules and the biological response.

4.3. Surface Chemistry

The surface of the delivery vehicle is one of the most important aspects of encapsulated drug delivery as the surface is generally the only interaction point with the body. If the surface is offensive to the body, the delivery vehicle is quickly recognized and removed via the immune system.[96] It is important to functionalize the surface of delivery vehicles to ensure that it is processed in the desired way. Adsorption of opsonin proteins can result in the delivery vehicle being engulfed by phagocytic cells of the immune system.[96] This reduces the circulation time of the delivery vehicle, prevents it from releasing its cargo in the necessary location, and may induce an immune response. Thus, measures should be taken to prevent this process.

Poly(ethyene glycol) (PEG) is a polymer typically used to prevent non-specific protein adsorption and improve blood circulation half-life. PEG attracts three water molecules per PEG residue, providing stealth properties and a steric shield, thereby minimizing non-specific protein adsorption.[97] Due to its low fouling nature, PEG does not interact strongly with other molecules and surfaces, so it is difficult to incorporate into LbL films. However, modification of PEG allows it to be included in films. A phospholipid derivative of PEG was adsorbed onto the surface of a fatty acid-modified multilayer through hydrophobic interactions.[98] The PEG coating was able to reduce adsorption of human serum albumin, and the binding and uptake of the particles by macrophage cells. Capsules functionalized for cell targeting used a PEG block copolymer to passivate the background surface.[82] In addition to the low-fouling PEG block, the block copolymer had a negatively charged PSS block to promote adsorption to particles with a terminating cationic layer. This surface resulted in a 5-fold improvement of specific to non-specific cell binding. An LbL film made entirely of PEG acrylate was synthesized using click chemistry.[99] This film prevented attachment and growth of cells on a glass slide, but

promoted growth when functionalized with an RGD peptide. This film highlights the diversity of materials that can now be incorporated into LbL films using novel assembly techniques.[100] Lipids may also be used to alter the surface chemistry of delivery vehicles. A lipid coating mimics the cellular membrane, and it may either enhance or reduce the interaction of the delivery vehicle with a cell. The use of charged lipids results in the formation of a lipid bilayer, whereas zwitterionic lipids form multilamellar structures.[101] A lipid coating decreases the permeability of the capsule, allowing the entrapment of small molecules without conjugation or sequestration.[101] The presence of the capsule provides structural rigidity and stability to the lipid layer. Moreover, the use of capsules predefines the size of the lipid coating, resulting in monodisperse populations.[102,103]

4.4. Intracellular Localization

The fate of most particles internalized by cells is processing through the late endosome and fusion with lysosomes. These vesicles have a significantly lower pH (4.5-5), and contain proteases and nucleases which are designed to degrade foreign material (Figure 6). Some delivery vehicles are designed to take advantage of the change in pH[104] or the presence of hydrolases to degrade or release their cargo; these are discussed in Section 6. Membranes were observed around polyelectrolyte nanotubes,[105] and also around large core-shell particles[88] that had been internalized into cells. The membranes suggest that internalized capsules are sequestered in lysosomal or endosomal compartments. A flow cytometric assay was used to determine if capsules were contained in the acidic compartments inside cells or if they were simply attached to the external membrane by encapsulating a pH sensitive dye.[106] This work suggests that at least a portion of the capsules were present in acidic compartments. Another investigation has revealed that dextran sulfate/poly-L-arginine capsules are only partially localized with lysosomes in the cell.[94] Protamine/dextran sulfate-coated 3 μm silica particles were observed in the cytoplasm without the surrounding membranes.[88] These

investigations suggest that at least some delivery vehicles were able to escape the lysosome. The “proton sponge” effect was proposed as a potential lysosome escape mechanism for the protamine/dextran sulfate particles.[88] Polyelectrolytes, particularly polycations with secondary and tertiary amine groups, promote endosomal escape through the “proton sponge” effect. The amine groups sequester protons in the lysosome, leading to a continued influx of protons, chloride ions and water into the lysosome. The resulting high osmotic pressure leads to membrane destabilization and endosome escape. This strategy is commonly used for DNA delivery and is one of the reasons that PEI is widely used. The use of a lysomotropic agent, which prevents acidification of lysosomes and results in their eventual destruction, greatly increased the effectiveness of cargo delivery.[88] This suggests that the particles are indeed internalized via a pathway that ends in fusion with the lysosomes and that in the future, further strategies are required to overcome this. Alternative methods for lysosome release are pH sensitive peptides[107] that are based on bacterial pore forming toxins[108] and viral peptides,[109] or synthetic polymers that are designed with the same goal.[110] A commonly used peptide for membrane disruption, TAT, has been incorporated into planar multilayered films, but has not yet been used for endosome/lysosome escape. TAT was incorporated in a multilayered film with DNA[47] and was delivered to cells grown on the surface of an exponentially growing film into which it was loaded.[111]

In these techniques for endosome/lysosome escape, the entire contents of the vesicle are expelled into the cytoplasm. This may lead to undesirable toxic side effects, such as the release of intracellular Ca^{2+} and activation of apoptosis proteins.[112] In the future, alternative methods for lysosome escape or avoidance may be required.

This section highlights the large number of variables that affect the way in which particles interact with cells. The physical and chemical characterization of the capsules becomes even more important when considering interactions in complex environments, such as those found in biology. The interconnectedness of the cellular pathways upon interaction with LbL

assembled materials is not well understood and requires further fundamental characterization in the future.

5. Cytotoxicity

Verification of the safety of nanomedicines is essential before clinical use can occur. The first step in this process generally involves in vitro experiments in cell culture, examining the toxicity of the material to the cells. Typically this is performed by a colorimetric method to measure the integrity of the plasma membrane or the cellular metabolism.[113]

Generally, cytotoxic studies of LbL assembled films and delivery vehicles have shown low toxicity, although this may in part result from the unreported investigations that eliminate or modify high toxicity samples. A selection of cellular viability investigations using LbL assembled materials is summarized in **Table 1**, and selected common polymers are listed in **Table 2**. Recent studies have attempted to understand the effect of delivery vehicles on living systems in more detail. For example, the viability studies performed on core-shell particles made with the common LbL polymers PEI, PAH, and dextran with rabbit mesenchymal stem cells were also supported by apoptosis and differentiation investigations.[114] Each of the experiments indicated that under the conditions used (0.1 mg mL^{-1}, 4 h incubation, 48 h growth period) there were no significant cytotoxic effects.[114] When considering cytotoxicity, the exposure time and concentration of the material are important parameters, as cytotoxicity generally increases with dose and time.[113] However, it is not clear if in vitro experiments can be used to predict in vivo behavior, or if any of the conditions listed in Table 1 accurately represent those expected during clinical treatment. For capsules injected in vivo, a moderate immune response was observed in histological sections, and for the same sample decreases in the in vitro viability measured using an MTT assay was observed with increasing concentrations of capsules.[115]

In addition to investigating the intact delivery vehicles, it is important to understand the cytotoxic effects of the polymers and degradation products used to assemble multilayered films (Table 2). This was performed for poly(β-amino ester)s used for DNA encapsulation and their bis(β-amino acid) degradation products.[116] Neither showed significant cytotoxicity under conditions in which PEI, a standard DNA delivery agent,[53] decreased the cell viability to below 20%.[116]

6. Degradation and Cargo Release

For drug delivery vehicles, cargo release is the primary objective. Cargo release can be achieved via diffusion or degradation. Cargo release via degradation can be dependent or independent of degradation of the delivery vehicle. For example, independent release occurs when a drug conjugated to the delivery vehicle via a peptide sequence may be cleaved by enzymatic degradation of the conjugating linker while the bulk of the delivery vehicle remains intact.[41] Most delivery vehicles are designed to be at least partially biodegradable (**Figure 8**).[117] This is to allow material excretion from the body and prevent toxic accumulation of foreign materials. The materials may be excreted via the renal or biliary pathways or metabolized. Materials that degrade into naturally occurring monomeric components may be metabolized by natural processes. For example, poly(lactic acid) nanoparticles were reduced to lactic acid and metabolized into carbon dioxide through the Krebs cycle.[118] Renal excretion is possible for polymers of less than 5000 g mol^{-1},[96] or for particles with a hydrodynamic radius less than 5.5 nm.[119] Larger particles may be excreted through the biliary system. In general, degradation studies of LbL synthesized materials are largely performed in vitro or in cell culture and have not yet addressed excretion. This is a future challenge for the application of these materials in vivo.

6.1. Optically Triggered Degradation

Near infrared (NIR) light is suitable for use in biomedical applications, as human tissue transmits light in the range 800-1200 nm. LbL capsules have been made sensitive to NIR light by the incorporation of gold nanoparticles into otherwise non-degradable multilayered films such as PSS/PAH.[120-122] When irradiated with NIR light, the gold heats to temperatures above the spinodal point of water, and the thermal stress resulting from the different thermal expansion coefficients of materials within the shell disrupts the film.[120] This technique has been used to trigger the release an active enzyme[120] and dextran[121] from capsules. Similarly, silver nanoparticles and an NIR absorbing dye were used instead of gold nanoparticles to effect the degradation of capsules under NIR light.[123,124] These capsules were internalized by cancer cells and demonstrated release of encapsulated dextran upon irradiation.[124] Light activated degradation allows both temporal and spatial control over cargo release, and is a promising mode of delivery. However, the limited penetration depth of the NIR light, which is on the order of microns depending on the conditions used,[125] may restrict widespread application of this technology.

6.2. Enzymatic Degradation

Enzymatically degradable films are generally made from polypeptides or polysaccharides, and they rely on the presence of appropriate enzymes for their degradation. This is a useful technique as proteases and nucleases are present in many specific areas of the body, which provides control over the timing or location of degradation. In particular, proteases are recruited to lysosomes to help destroy foreign compounds or cellular waste. Many delivery vehicles enter the lysosome on internalization; and so this enables the drug to be released at this point. Planar polysaccharide films made from LbL assembly of chitosan and hyaluoronic acid were degraded in vitro by the enzymes lysozyme and hyaluronidase.[126] The films were made resistant to enzymatic degradation by covalent crosslinking. Additionally, increasing the

molecular weight of the building blocks to assemble the film increased resistance to enzymatic degradation. Macrophage cells were seeded onto the films and after 24 h, some degradation had occurred. Finally, the films were placed in the peritoneal cavity of a mouse for 6 days. The crosslinked films demonstrated increased resistance to degradation in vivo compared to non-crosslinked films.

Similarly, a film created from the PLL and DNA demonstrated tunable degradation by the incorporation of crosslinks formed by gluteraldehyde.[127] Trypsin degraded the PLL, resulting in DNA release. The crosslinking density was modified by altering the time in the gluteraldehyde solution. Increased crosslinking time (from 3 h to more than 24 h) resulted in slower DNA release. In another DNA-containing film, it was the DNA that was enzymatically degraded to release a synthetic polycation poly(diallyldimethylammonium chloride).[128] The enzyme, DNase I, cleaved the DNA at the surface of the film and resulted in stepwise degradation of the multilayered film. The rate of degradation was controlled by altering the activity of DNase I with the concentration of the ions Mg^{2+} and Ca^{2+}. Capsules assembled entirely from DNA have also been shown to degrade specifically in the presence of restriction enzymes. The films are held together based on the hybridization of base pairs, and restriction enzyme cut-sites can be engineered into the oligonucleotides used in the assembly.[129] Capsules which contained an EcoRI cut-site degraded in the presence of the EcoRI enzyme, whereas capsules that lacked this sequence did not. These DNA capsules can also be non-specifically degraded by non-specific nucleases such as DNase I. Resistance to non-specific nucleases can be induced by incorporating peptide nucleic acids (PNA).[130]

Decher and colleagues have recently attached DOX to a film via an enzyme degradable linker;[41] this represents an example where the cargo release is independent of the degradation of the film. Approximately 50% of the DOX was released in 24 h when the linker contained a specific amino acid sequence, whereas the release was negligible for a linker with a random sequence (**Figure 7**).

Enzymatically degradable carriers receive much interest because of the wide range of polymers that can be incorporated, resulting in carriers with a variety of properties. Additionally, these films will eventually be degraded to monomeric components and excreted or metabolized. However, enzymatically degradable carriers are not ideal for all types of cargo. The cargo should be resistant to the enzymes and conditions used for its release. However, the recruitment of enzymes was recently used to degrade released cargo that fluoresced upon degradation, signaling the successful internalization, degradation and release of the cargo inside cells.[131] The dextran sulfate/poly-L-arginine capsules were degraded and released DQ™-ovalbumin, a protein that fluoresces when it is degraded. These capsules show promise for the delivery of vaccine antigens that require intracellular processing, such as degradation and subsequent presentation to immune cells. The capsules were successfully used to selectively activate Th17 responses in vivo, which are involved in the induction of immunity against bacteria and fungi.[94,132]

6.3. Hydrolytic Degradation

Some multilayered films are designed to degrade gradually in physiological conditions by hydrolytic cleavage of the polymer backbone. An example of such a polymer is a poly(β-amino ester), which is a cationic poly(amine) containing hydrolytically degradable esters along the backbone.[116] Polymers from this class were incorporated in LbL films by alternation with PSS,[133-137] polysaccharides,[138,139] pDNA,[45,72,140,141] and the proteins ovalbumin[69] and lysozyme.[142] Films formed from poly(β-amino ester)s are some of the best understood and most promising LbL assembled films for drug delivery. They have been investigated for gene delivery,[72,140] DNA vaccines,[50] and vaccine antigens.[69] LbL assembly of the poly(β-amino ester) is typically performed at a mildly acidic pH (< pH 5), where the hydrolysis of the polymer is very slow. The degradation rate of the film in different pH can be predicted using a pseudo first-order kinetic model.[138] In physiological conditions the

hydrolysis of the polymer is increased, causing polymer fragmentation. The affinity of the small molecular weight fragments to the polyanion layers was reduced, resulting in defoliation of the film. When placed in an isotonic buffer, phosphate buffered saline (PBS), systematic variation in the charge density and hydrophobicity of poly(β-amino ester)s caused the duration of film erosion to increase,[134,139] although further increases resulted in rapid film destabilization with an alternative mechanism.[139] The degradation was further tuned by using combinations of the different polymers in the thin film, although the erosion rate was largely influenced by the most hydrophobic poly(β-amino ester).[135] The mechanism of film degradation is likely to be surface erosion, and was gradual and uniform.[136] Further investigation confirmed that polymer hydrolysis was important for film erosion.[137]

Novel hydrolytic charge-shifting polymers have also been incorporated into LbL films,[48,85,143,144] and have been able to extend the duration of film erosion beyond that obtainable by hydrolytically degradable polymers. Charge-shifting polymers undergo dynamic reactions that alter their net charge. One such polymer had a poly(acrylamide) backbone and pendent tertiary amines connected through an ester bond. The ester was hydrolyzed over time to leave pendent carboxylic acids; which resulted in a shift from positive to negative charge. In their positively charged form they were alternated with pDNA to form a multilayered film.[144] After hydrolysis of the pendant cationic groups, the negatively charged polymer repelled the pDNA, resulting in pDNA release. After an initial delay of 25 days, pDNA was released from the film over 90 days. Similarly, a cationic amine-containing polymer functionalized with ester “charge shifting” side chains was incorporated into a multilayered film with pDNA.[48] This polymer resulted in release of pDNA over 5 days. The erosion mechanism of this system is likely to be top-down surface erosion, as two pDNA constructs embedded in different layers of the film were released sequentially. An anionic charge-shifting polymer was created to release the cationic component of a multilayered film.[143] PAH was modified with a citraconic anhydride to form a negatively

charged polymer that charge-shifted in acidic pH to revert back to PAH. An LbL film consisting of PAH and charge-shifting modified PAH released PAH over 4 days at pH 5.

In an alternative approach, polymer capsules can be broken provided there is enough stress to rupture them. PSS/PAH films have been formed around hydrolytically degradable, dextran-based microgel particles.[145] The microgel particles swelled as they were hydrolyzed at pH 9, causing the thin film to rupture when 10% of the microgel had degraded. However, the film remained intact when the microgel was degraded at pH 7 as the microgel fragments diffused through the film. More recently, dextran sulfate/poly-L-arginine films were assembled around 150 μm diameter biodegradable microgels.[146] The microgels were loaded with 50 nm polystyrene particles, which exhibited burst-release upon degradation of the microgel core. The release occurred after 20 days at 37 ºC and could be controlled by altering the cross-linking density of the core particle. The integrity of the membrane was investigated after injection into the flanks of mice. Most particles appear intact after 14 days; however after 30 days most appear broken. These particles are being investigated for delayed vaccine delivery.

6.4. Redox-Activated Degradation

Disulfide bonds are easily formed by oxidation of thiol groups and are easily cleaved by the reverse process (reduction). Reducing environments occur at specific locations in the body (the cytosol of the cell and the colon[147]) whereas other locations are comparatively oxidizing (e.g., the blood stream). Thus, redox-activated degradation is “triggered” on cellular internalization, as it does not occur in the comparatively oxidizing environment of the blood stream or during storage in physiological buffers. For LbL films, disulfide bonds may be included either along the backbone of the polymers or as crosslinks between subsequent polymer layers. However, there is an additional requirement for degradation: the film without crosslinks must be inherently unstable.

The first examples of reductively degradable films were formed by the LbL of positively and negatively charged peptides containing cysteine residues.[148,149] After electrostatic film assembly, the thiol groups of the cysteine residues were oxidized to form disulfide crosslinks between layers. These films were reductively degradable under acidic conditions, where the electrostatic interactions were disrupted. The negatively charged component was uncharged below its pK_a and the repulsion of the remaining positively charged peptides resulted in deconstruction of the reduced film. The degradation of the film was controlled by altering the concentration of the reducing agent dithiothreitol (DTT).[148] At the highest DTT concentration tested (13.33 mM), only 15% of the capsules remained after 8 h. In a similar approach, a PMA/poly(vinylpyrrolidone) (PVPON) hydrogen-bonded film was stabilized by inter-layer disulfide bonds between alternate PMA layers. The film is formed by hydrogen bonding at low pH where the PMA is protonated. After film formation, the thiol groups on the thiol-functionalized PMA are oxidized to form crosslinking disulfide bonds. These bonds are required to stabilize the film at physiological pH, when the ionization of PMA destroys the hydrogen bonding. In a reducing environment the disulfide crosslinks are reduced to thiols, resulting in destruction of the film. This film was demonstrated to swell and then degrade in approximately 4 h when placed in a cellular concentration of glutathione (GSH; 5 mM GSH in PBS at 37 ºC), whereas capsules in PBS remained intact.[150] GSH is the most important non-protein thiol source in the cell and is used to maintain the redox potential,[151] and thus is an appropriate reducing agent to mimic cellular conditions. Further investigations showed that the degradation rate could be controlled by altering the degree of thiol modification along the PMA backbone.[152] However, increasing the thickness of the films did not alter the degradation rate, indicating a bulk-type erosion mechanism.[152] Oligopeptide cargo conjugated to PMA though a disulfide bond was released within 2 h,[153] indicating that the cargo can escape partially degraded capsules. These capsules have promise for vaccine delivery, [79,153,154] cancer therapy,[37] DNA encapsulation,[21,22] and as microreactors.[51,62]

Low fouling, redox-active capsules were created by crosslinking PVPON with a click disulfide crosslinker.[155] These capsules were degraded within 3 h in 5mM GSH at 37 ºC, whereas capsules in PBS were stable.

In an alternative approach, disulfide bonds were incorporated into a polymer backbone. A cationic polymer was synthesized by the oxidative polycondensation of TAT, a short positively charged polypeptide derived from the transcriptional activator protein of HIV-1, which was modified to contain flanking cysteine residues.[47] A thin film was created by performing LbL with the disulfide-containing polymer and pDNA. When the polymer was reduced, the resulting low molecular weight peptides displayed a decreased affinity to the DNA under high salt conditions; which resulted in film deconstruction over 24 h. Similarly, a poly(amidoamine) containing disulfide bonds along the backbone was used to form a planar multilayered film with pDNA.[156,157] The rate of degradation was controlled by the concentration of the reducing agent DTT.[157] In 5 mM DTT, the film deconstructed in a few hours, whereas it took 9 days to deconstruct in 1 mM DTT.

7. Summary and Outlook

LbL assembly of polymers into multilayered films is promising for the delivery of drugs, both from the surface of macroscopic objects and as colloidal carriers. Perhaps the biggest advantage of this technique is its versatility. Each application requires particularly designed specifications, and the LbL technique allows the surface chemistry, size, permeability, and degradability to be altered to suit the application. It is clear from this review that films can be created to practically any specification. Highly specialized films have been created in recent years. Many different types of drugs can be encapsulated efficiently, either into the film or into the cavity of the carrier. Additionally, multiple drugs can be incorporated into the same carrier. The surface chemistry is easily altered by adding layers of different polymers or attaching targeting moieties. The degradation and cargo release can be finely controlled by a variety of mechanisms. However, the implications of these material designs in biological

environments are less well understood and less frequently investigated. Biodistribution studies are urgently required if these materials are to be considered for drug delivery in the near future. Additionally, the internalization and effect of the materials, as well as their therapeutics on cells and organs, require additional characterization and optimization. Materials are already being assessed by biological feedback to promote rational design of ever more complex carriers. Investigations of this sort are likely to be more common in the future, and are essential if we are to see FDA-approved products based on LbL assembly.

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Biographies

Dr. Alisa L. Becker has recently completed her PhD on the use of multilayered polymeric capsules for nucleic acid delivery at the Department of Chemical and Biomolecular Engineering, The University of Melbourne, under the supervision of Prof. Frank Caruso. Her research interests include the design and characterization of nanoengineered materials for use in biological applications.

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Prof. Frank Caruso received his PhD degree in 1994 from The University of Melbourne, and then moved to the CSIRO Division of Chemicals and Polymers in Melbourne studying the interfacial alignment of receptor molecules for biosensor applications. He was an Alexander von Humboldt Research Fellow and then group leader at the Max Planck Institute of Colloids and Interfaces (Berlin, Germany) from 1997-2002. Since 2003 he has been a professor, an Australian Research Council Federation Fellow, and heads the Centre for Nanoscience and Nanotechnology in the Department of Chemical and Biomolecular Engineering at The University of Melbourne. His research focuses on polymers at interfaces, nanostructured colloidal systems, nanocomposite thin films, and biomaterials.

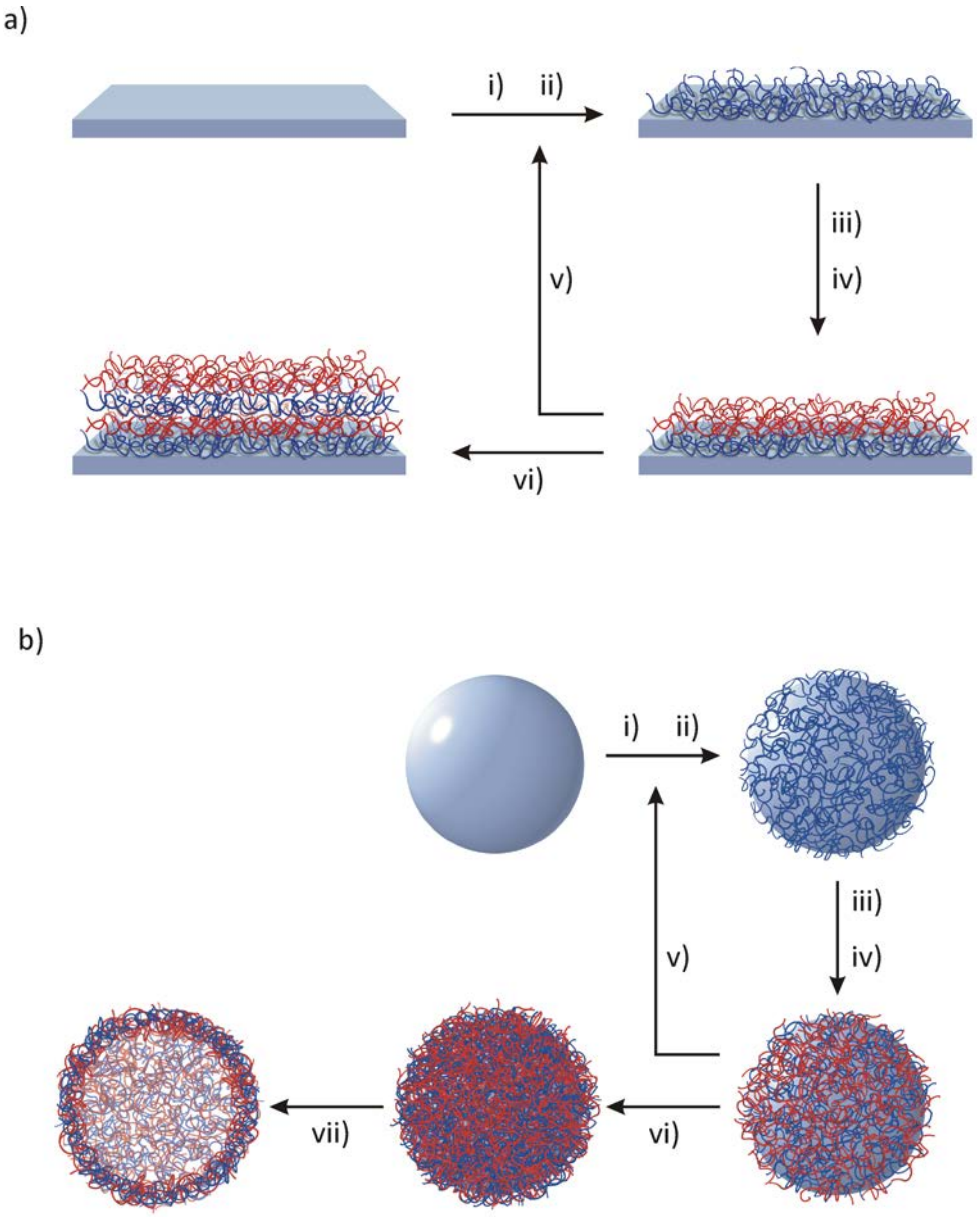


Figure 1. LbL assembly on a) planar and b) colloidal substrates. i) Immersion in a positively charged polyelectrolyte solution; ii) Rinse; iii) Immersion in negatively charged polyelectrolyte; iv) Rinse; v) Repeat i)-iv) until desired number of layers has been deposited; vi) Core-shell particles can be the end of the process or vii) the template is dissolved away to leave a hollow polyelectrolyte capsule.

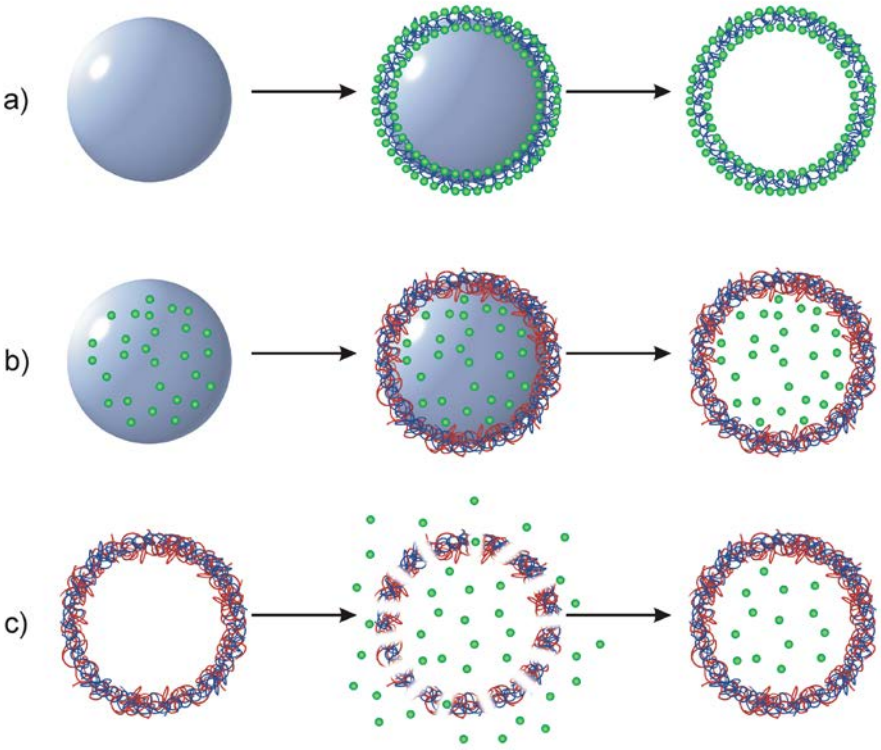


Figure 2. Typical methods for cargo encapsulation within LbL capsules. a) Use of the cargo as a constituent of the capsule wall. b) Pre-loading the template with the cargo prior to LbL thin film formation. c) Post-loading the capsule by altering the permeability of the thin film and entrapping the cargo inside.

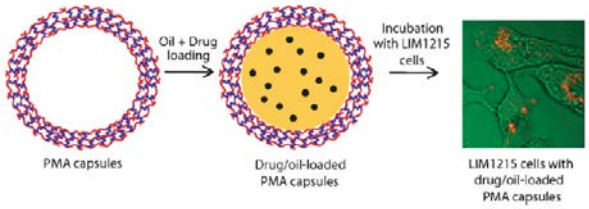


Figure 3. Encapsulation of DOX into polyelectrolyte-coated emulsions for redox-triggered release of DOX inside cancer cells. Reproduced with permission from Reference [37]. Copyright 2009, Wiley.

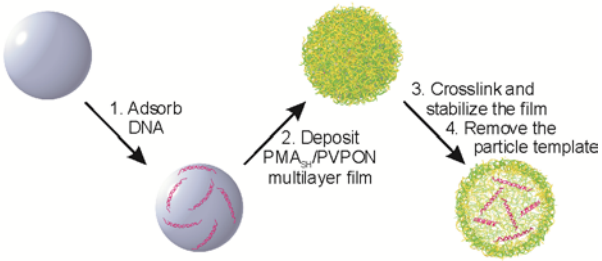


Figure 4. Encapsulation of DNA within LbL assembled capsules using the pre-loading template method. Reproduced with permission from Reference [21]. Copyright 2008, American Chemical Society.

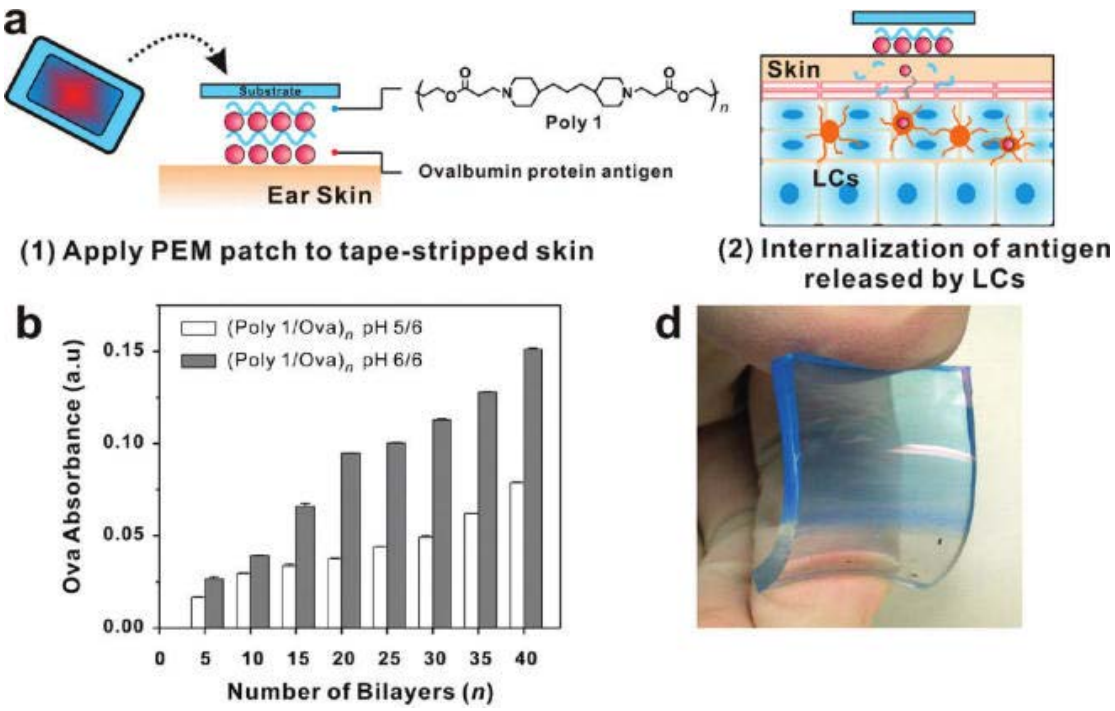


Figure 5. Hydrolytically degradable thin films incorporating a vaccine antigen were assembled on a flexible PDMS substrate for transdermal vaccine delivery. Reproduced with permission from Reference [69]. Copyright 2009, American Chemical Society.

Figure 6. Pathway for successful drug delivery: i) The delivery vehicle approaches the cell and is internalized by endocytosis into ii) an endosomal or lysosomal vesicle. iii) The vesicle is ruptured or iv) the delivery vehicle escapes from the vesicle into the cytosol. The drug may be still encapsulated and require v) release from the delivery vehicle. The drug may be active in the cytosol or may require vi) further transport into the nucleus or other organelles.

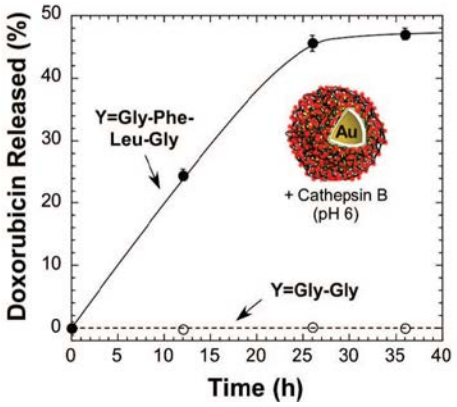


Figure 7. Release profile of DOX during incubation with the enzyme Cathepsin B. Specific cleavage of the peptide (Y) Gly-Phe-Leu-Gly spacer between DOX and the polymer backbone. The control with a (Y) Gly-Gly spacer is not cleaved. Reproduced with permission from Reference [41]. Copyright 2009, American Chemical Society.

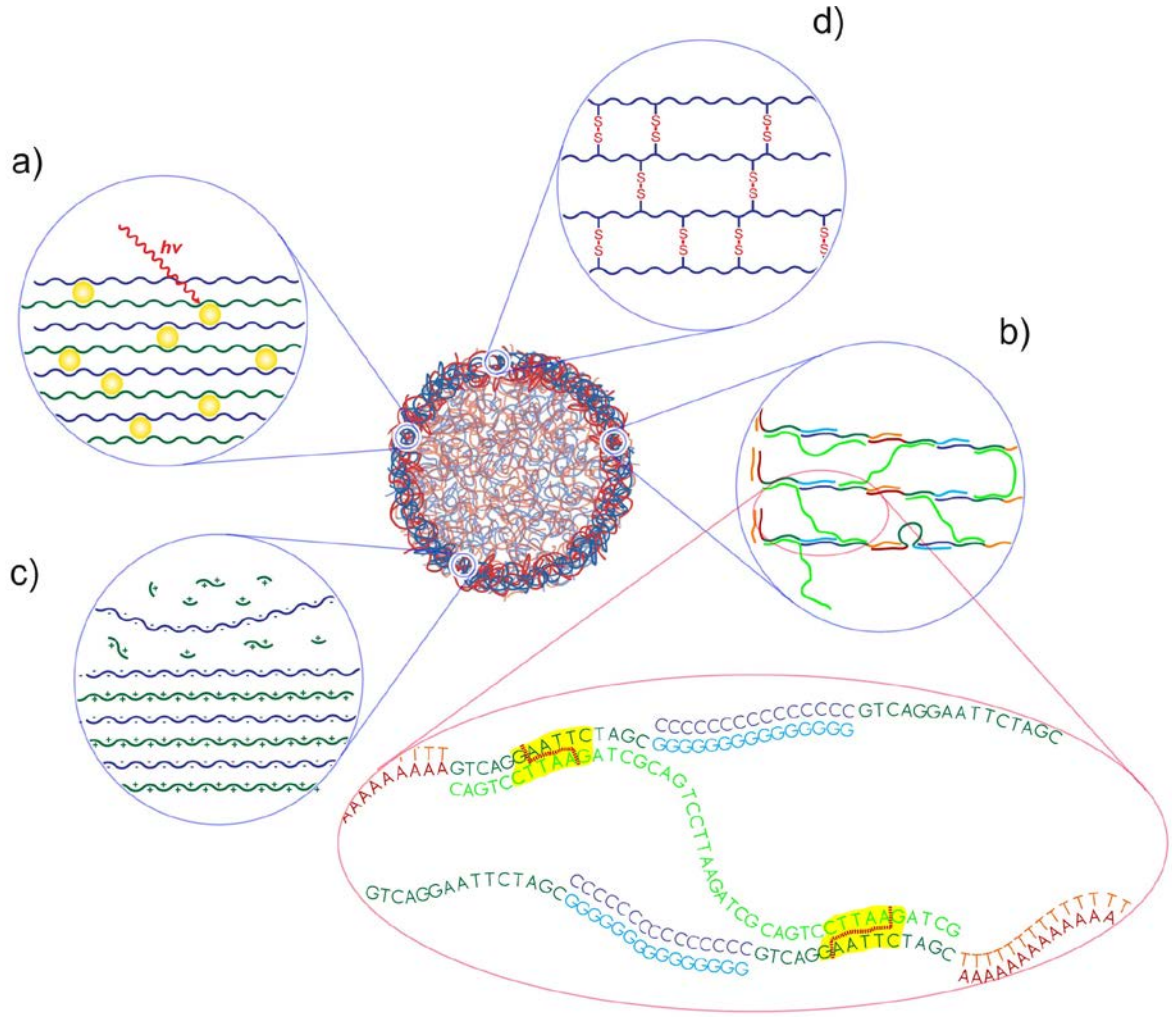


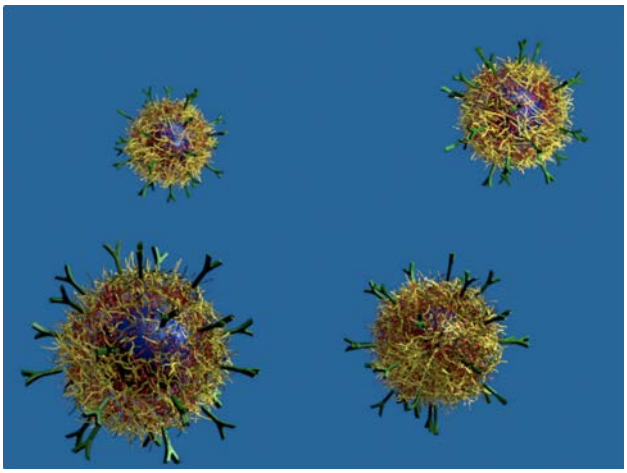
Figure 8. Degradation mechanisms for LbL assembled capsules (these are also suitable for thin films on planar surfaces) a) optically triggered degradation, b) enzymatic degradation, c) hydrolytic degradation, d) redox-activated degradation.

Multilayered polymeric capsules and films formed using the layer-by-layer technique are promising for next-generation therapeutic delivery. This review discusses important and recent contributions in the use of these materials for the delivery of a wide range of therapeutics. It highlights their advantages and notes areas for further investigation.

Drug delivery

A. L. Becker, A. P. R. Johnston, and F. Caruso*

Layer-by-Layer Assembled Capsules and Films for Therapeutic Delivery



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