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Recent Developments in Multilayered Polymeric Particles – From Fabrication Techniques to Therapeutic Formulations

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Multilayered particles are emerging as a powerful platform in pharmaceutics, especially for targeted, triggered and sustained drug delivery. These novel delivery systems exhibit advantages over single-layered particles, such as mitigating burst release of drugs. In this article, an overview of recent developments in the fabrication of multilayered polymeric particles, towards their utilization in therapeutic applications, will be reviewed. Fabrication techniques that aid in engineering multilayered particles will first be discussed. Towards the end, a critical outlook of the key issues associated with this particulate delivery system will be addressed.

1. Introduction

Several decades ago, the power of encapsulation was realized and employed to store different solids or liquids, protecting delicate substances from environmental influences. Nowadays, encapsulated materials are widely used in the pharmaceutical, cosmetic, food, textile, adhesive, and agricultural industries. With the rise of nanotechnology, engineering of functional structures at the nanoscale becomes feasible, and a wide range of particles, including inorganic (e.g. gold, silica, zinc oxide, silver, etc.) and organic (e.g. liposomes, polymeric, etc.), have been generated and utilized for encapsulation. These particulate systems complement well with pharmaceutics as they facilitate drug encapsulation, while providing extraordinary sensitivity and specificity for diagnostic and therapeutic interventions.[1] Designing a particle that can act as a drug delivery vehicle can therefore be challenging as one has to aim for a robust and versatile system where hydrophobic, hydrophilic and/or amphiphilic drugs have to be encapsulated, delivered only to the diseased tissue, and released in a controlled manner. In addition, if a stimulus-responsive release is desired, the carrier has to be designed to react to a specific internal or external stimulus. Such delivery systems are thus envisaged to improve treatment efficacy, with fewer adverse effects, thus improving patient compliance.

In this regard, multilayered polymer particles with tailorable properties are an excellent choice. These particles can be fabricated by layering various polymers onto substrates (i.e. nano / micro-particles, etc.).[2, 3] For example, core-shell particles can be generated by layering onto a sacrificial template to generate hollow-shell particles (i.e. capsules).[4, 5] With a wide range of layering techniques, materials, and templates available (including inorganic particles[6]), multilayered particles now can be designed and tailored to be a powerful platform for theranostics applications[7]. The application of multilayered particles as therapeutics is therefore rapidly emerging, with significant developments in recent years. As an initial example, a recent study of dual drug-loaded multilayered particles on three-dimensional (3D) MCF-7 spheroids[8] demonstrated a greater reduction in tumor growth rate, while another study showed capability of tumor targeting[9]. In this paper, an overview of the recent advances in the fabrication and formulation of multilayered polymeric particles for therapeutic applications will be reviewed.

2. Fabrication Techniques of Multilayered Particles

2.1 Layer-by-layer (LbL) assembly

Over the past few years, a number of fabrication methods have been devised to prepare multilayered particles, as well as capsules.[10-12] An influential domain in the use of non-covalent interactions is LbL assembly. In addition to electrostatic interactions, which is based on the absorption of alternating cationic and anionic species, other complementary interactions include hydrogen bonding,**Error! Bookmark not defined.** hydrophobic interactions,[13] covalent bonding,[14] DNA hybridization,[15] van der Waals,[16] and guest−host interactions.[17] This technique enables the engineering of multilayered particles with a range of tunable properties, i.e. stiffness, permeability, biofunctionalization and biodegradability. The popularity of this method lies in its simple, inexpensive, and readily accessible process, and the ease in creating highly tailored polymer layers with a range of functionalities. Here, we will discuss further on LbL formations via electrostatic, H-bonding and covalent bonding.

(a) Electrostatic interaction

Particles with polymeric multilayers are typically assembled through a sequential deposition of charged polymers via complementary interactions onto a template or substrate[4, 5]. Briefly, electrostatic interactions between polyions in solution and an oppositely charged

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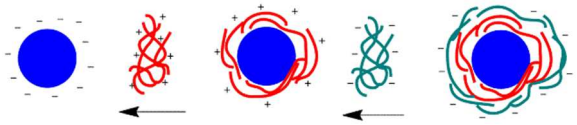
interface are key to the final structure of the polyion-layered thin film (**Scheme 1**). During the self-assembly process, polymer adsorption continues until the surface charge is reversed so that the adsorption of a counter-polyion proceeds in a next step. In this manner, the alternating layers of polycations and polyanions on colloidal particles can be deposited uniformly and repeatedly. The final polyelectrolyte-based multilayered particle consists of layers with nanometer thickness. Sometimes, it is also possible to remove the colloidal core to produce hollow capsules instead of solid particles. Besides this, in a few other cases, drug crystals themselves can act as core materials for the layering of polyelectrolytes.[18, 19]

Majority of this work revolves the use of fully or nearly fully-charged polyelectrolytes to avoid flocculation tendency of particles; a problem observed during assembly process when coating with partially-charged or weakly-charged polymers[20]. However, weak polyelectrolyte multilayers of low charge density have been shown to be practically useful in inducing reversible porosity transitions, discontinuous swelling transitions, and nanoreactor capabilities suitable for *in situ* fabrication of nanoparticles[21-24] To overcome this, Schuetz et al. used copper assisted LbL deposition, working on poly(acrylic acid) and poly-(allylamine hydrochloride).[25] It is also evident from Rubner et al. that excellent long-term cell adhesion resistance is possible with weak polyelectrolyte multilayers.[26, 27] Hence, the ability to create colloidal particle coatings with certain weak polyelectrolyte multilayers, of particular functional groups, is clearly a desirable goal. By using a small number of layers adsorbed from salt solutions, Burke et al. were also able to demonstrate weak polyelectrolyte multilayer coatings on colloidal particles.[28]



Scheme 1. Schematic diagram of the build-up of multilayer assemblies on spherical particles by consecutive adsorption of anionic and cationic polyelectrolytes. This diagram is highly idealized. In reality, the layers are well interpenetrated. Adapted from webpage: http://www.pharmafocusasia.com/research_development/layer-by-layer-micro-nano-drug-encapsulation-eolyelectrolytes.html

(b) H-bonding

In addition to electrostatic interaction, H-bonding is also exploited for the formation of multilayered particles[11]. For example, capsules comprising alternating layers of neutral polymers such as poly-N-vinylpyrrolidone or polyethylene oxide and polymethacrylic acid can be prepared onto cadmium carbonate core using this technique. To increase layer adhesion, cadmium carbonate particles were pre-treated with polymethacrylic acid at pH 7.0, followed by washing with buffer at pH 3.5, so as to elicit H-bonding; giving a composite capsule that has significant stability.[29] It should be noted that the first report of H-bonded multilayered capsules involved the assembly of poly-(vinylpyrrolidone) and m-methylphenol-formaldehyde resin from methanol solutions.[30] In their recent work, Chen et al. reported a new method to synthesize multilayered polymer capsules with hemispherical concave, spherical, and cubical geometries[31]. These robust monodispersed particles were prepared from their corresponding spherical and cubical multilayer capsules of poly(N-vinylpyrrolidone)/tannic acid (PVPON/TA)n (n>15; n= number of bilayers) via H-bonding (**Figure 1**). Layers such as PVPON and TA were selected due to their ability to form stable H-bonded multilayered films over a wide range of pH.[32-34] These layers were first deposited onto spherical SiO_2 or cubical $MnCO_3$ cores before dissolving the core to give a hollow capsule. They have also demonstrated an interesting phenomenon that H-bonded hollow particles preserve their original shapes in their dry states, a contrast to other reports. It is shown that the capsule thickness-to-shell size ratio (correlating to capsule stiffness) determines shape transformation or retention of the particle. For example, when this ratio is between 0.041 and 0.055, hemispherical particles are obtained from spherical capsules. On the contrary, dried cubical and spherical hollow particles can be obtained from (PVPON/TA)n when $n \geq 25$. Upon re-hydration, the newly formed particles retained their shapes.

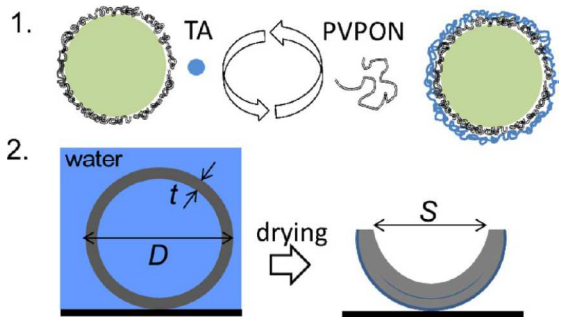


Figure 1. (PVPON/TA)n multilayers were formed on spherical SiO_2 cores using H-bonded LbL assembly (1). After core dissolution, spherical capsules, with shell diameter of D and the shell thickness of t, were drop-cast on Si wafers and dried at room temperature to result in hemispherical concave particles with the opening size of S (2).[31]

In another study, Yang et al. demonstrated that H-bonded bioinert multilayer coatings containing poly(acrylic acid), a weak polyelectrolyte, can be assembled onto colloidal surfaces, and subsequently stabilized by crosslinking via simple carbodiimide chemistry.[35] Carbodiimide chemistry has been previously used to crosslink electrostatically assembled multilayers.[25,36, 37] More recently, Kozlovskaya et al. reported an approach to create stable H-bonded multilayers on colloidal particles,[38] whereby carboxylic acid groups were activated with a carbodiimide, and an additional difunctional agent was added to promote crosslinking. This produces a semi-interpenetrating polymer network that stabilizes the multilayer complex at higher pH.[38] In their work, Yang et al.[35] showed that H-bonded multilayer films comprising of poly(acrylic acid) (PAA) and polyacrylamide (PAAm) can be stabilized at high-pH conditions using carbodiimide chemistry without the need for a crosslinking agent. The resultant multilayer coatings are stable at physiological pH, cell culture media and exhibit the ability to resist cell attachment. The availability of reactive carboxylic acid groups makes it possible to further functionalize the coatings with cell-binding ligands (i.e. RGD) or metallic nanoparticles with antibacterial properties, i.e. silver.

(c) Covalent bonding: Layering by 'grafting from' approach

An alternative method to achieve multilayered particles with a well-defined core-shell morphology is to graft polymer brushes through the use of initiators tethered chemically to the particle surface.[39-47] Yang et al.[48, 49] explored the possibility of using a two-step process to prepare core-shell polymer particles. The initiators were immobilized by a photo process and reactivated by a thermal process. For example, the immobilization of the dormant species onto spherical particles of vulcanized NBR (acrylonitrile butadiene rubber) latex by a photo process consist of three steps, as depicted in **Scheme 2**: (1) Benzophenone (BP) and a small amount of tri-functional monomer trimethylpropane triacrylate (TMPTA) were

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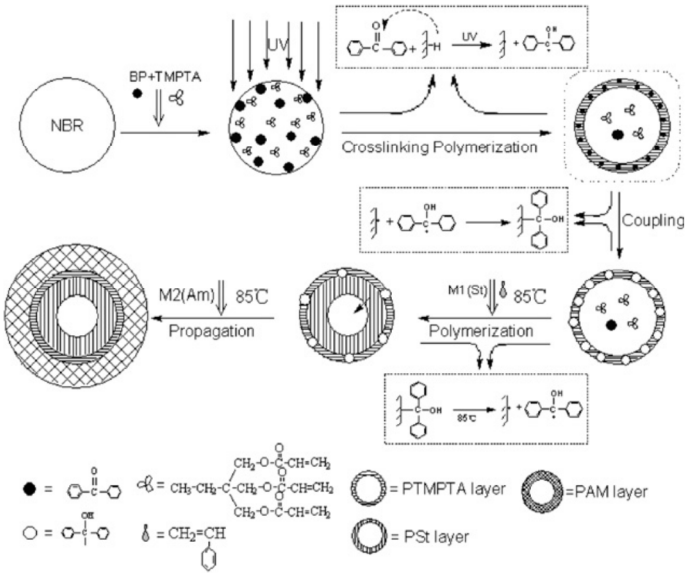
added into NBR latex; (2) under UV radiation, a free radical crosslinking polymerization on surface/subsurface was initiated by BP photoreduction; (3) free radical coupling reaction with the semipinacol radicals. Due to the crosslinking reaction of TMPTA, surface coupling of semipinacol groups becomes favourable. Following this, particles with covalently connected initiators were used to initiate the grafting polymerization via heating as shown in **Scheme 2**. A high surface grafting efficiency of ~90% was achieved with a low amount of homopolymer in solution. This is possible due to the very low initiation reactivity of the semipinacol radicals produced by the decomposition of NBR-SP at 85 ºC in the heterogeneous polymerization system. This ''grafting from'' technique achieves covalently bonded stable layers of densely grafted polymers with a variety of compositions and functionalities on the surfaces of micro/nanoparticles such as latexes, gold, silica, etc.



Scheme 2. Schematic diagram showing procedure and chemistry for grafting polymerization on the surface of NBR latex particles.[49]

2.2. Mechanized Fabrication Techniques

Despite considerable progress in the field of polymeric multilayering, the sequential layering process is often labour intensive and time consuming. Centrifugation is often performed after each deposition cycle, but this is not easily automated, thus requiring significant effort and time, especially if numerous layers are desired. For nanoparticles, aggregation is another challenging issue. Although there are reports of multilayered gold nanoparticles[50] and quantum dots[9], generating multilayered particles with lower-density templates, such as silica or polymer nanoparticles, is challenging. In summary, the advantages LBL assembly are as such:

Advantages:
- Good chemical stability for short term
- Wide size range, but low dispersity
- Controllable permeability of drugs
- Predictable/tunable surface chemistry
- Multifunctional/multivalent patchy surfaces possible
- Modification of core and shell – both possible and hence the properties
- FDA approved polymers can be usable

However, the disadvantages of LBL technique are also clearly evident as listed below:

Disadvantages:
- Time-consuming and labor intensive preparation process
- Poor yield
- Poor reproducibility
- Permeable to small drug molecules
- Aggregation issues
- Long-term stability issues unlike covalently linked system

To address some of these challenges, several alternative methods to assembly multilayered particles, through mechanical instruments, have been developed, including fluidized bed coating[51, 52], spray coating,[53] surface acoustic wave atomization,[54] membrane filtration,[55] and microfluidic methods.[56, 57] In fluidized bed, coating of solid particles happens inside the bed, and a layer is deposited onto solid particles by a coating solution. At the same time, the fluidizing gas dries the coating layer.[52] Another method is spray coating, whereby it involves the spraying of the coating solution onto the particles embedded in a mesh.[53] In the atomization technique, a template-free approach is used to produce nanometer-sized multilayered particles, but the absence of a template limits the diversity of particles that can be synthesized by standard centrifugation methods.[54]**Error! Bookmark not defined.** For membrane filtration, polymers can be sequentially and continuously added to a particle suspension and removed through membrane filtration. However, filter caking is an imminent issue here.[55] Lastly, in microfluidics, the assembly process can be controlled with a high level of precision.[56, 57] Recently, Priest et al.[56] fabricated a microfluidic system where layer build-up was achieved by sequential inclusion and withdrawal of polymer solutions into a continuous flow of templates. Kantak et al.[57] reported a microfluidic pinball method to fabricate multilayered capsules and have shown that layer build-up can be achieved by physically displacing the templates back and forth between the washing and polymer solutions using adjacent laminar flows of these solutions within a microfluidic system. Both methods can be utilized for the continuous production of particles with minimal labour and time, but scaling up would be challenging due to the inherently small dimensions of microfluidic channels.

(a) Electrophoretic Polymer Assembly (EPA)

More recently, Richardson et al. developed an interesting microfluidic system called "electrophoretic polymer assembly".[58] Here, they first immobilized the nanometer- or micrometer-sized templates (silica nanoparticles) inside an agarose gel before polyelectrolytes were injected and allowed to pass through the gel; a technique analogous to standard DNA gel electrophoresis (**Figure** 2). After several cycles of polymer injection, melting of the agarose recovers the multilayered particles. Here, they have successfully fabricated stable LbL films onto 35 nm silica nanoparticles, which is otherwise not easily achievable by conventional centrifugation method.

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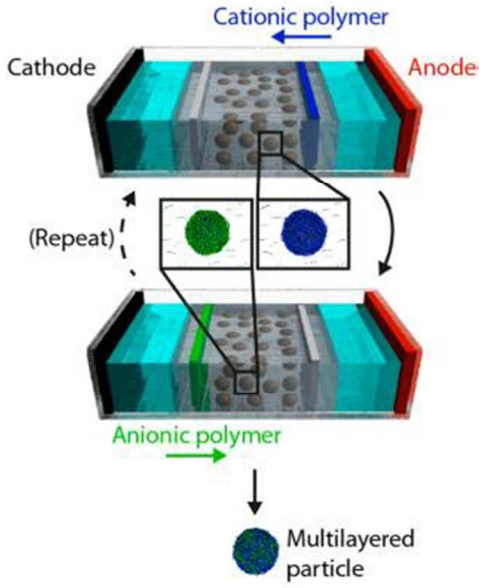
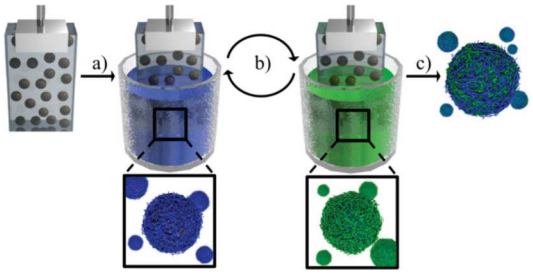


Figure 2. Electrophoretic Polymer Assembly: multiple polymer thin films were assembled onto particles immobilized in agarose and layering happens by moving the polymer using electrophoresis[58]

Although electrophoretic polymer assembly (EPA) is useful for preparing a range of LbL assembled capsules with desired material properties and imparts advantages such as short hands-on time, amenability to automation, and simplified use of small templates, it has some stringent requirements: (i) the need for a dissolvable rous immobilizing matrix, and (ii) materials used must move under electrophoresis or induced electro-osmic flow. Uncharged polymers are therefore challenging to electrophorese, and EPA cannot be applied universally to a full range of materials and functionalities.

(b) Immersive Polymer Assembly (IPA)

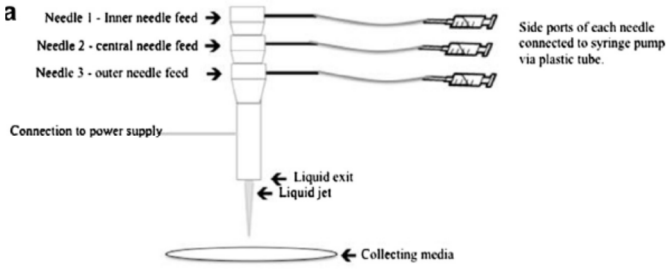
Nonetheless, these problems can be circumvented by an approach called immersive polymer assembly (IPA) introduced by Richardson et.al.[59] They have reported a method to prepare multilayered polymer capsules based on layering of immobilized particle templates that is inherent to uncharged polymers. This allows for parallel layering of different particles and/or polymers, and is easily automatable. Immobilizing templates or sacrificial particles in a gel essentially simplifies the complexity of layering onto 3D substrates as it can be treated as a 2D planar scenario. Practically, IPA can use standard, planar dip-layering protocols on immobilized particles with little modification. Immersing the gel-immobilized particles in polymer solution results in polymer assembly on the particle surface similar to LbL assembly on planar substrates. After recovery and template removal, stable capsules of different diameters are obtained (**Scheme 3**). IPA can be easily automated by using it with a StratoSequence robotic dipper (Nanostrata Inc.) as exemplified by Dubas et.al.[60, 61]



Scheme 3 Template particle coating via IPA: a) Immobilized particles are immersed into a polymer solution, followed by washing steps (in water). b) Subsequent immersion into solution of a complementary polymer, followed by water washing. Process is repeated until the desired number of layers is attained. c) The layered particles are recovered from the agarose, and the template particles can be dissolved to yield capsules.[59]

(c) Electrohydrodynamic jetting

In a recent study, Mohan et al demonstrated the feasibility of using an electrohydrodynamic process with a triple needle device to prepare nearly mono-dispersed, spherical, tri-layered sub-micron particles (**Scheme 4**).[62] Three biocompatible polymer solutions of poly (lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL) and polymethylsilsesquioxane (PMSQ) were used to prepare around 300 nm spherical particles with three distinctive layers. Particles were proven to be non-cytotoxic, indicating their potential suitability for medical applications. The ability to fabricate such multilayered particles in a single step, under ambient conditions has considerable potential for a range of applications in particular controlled release drug delivery system with multiple loading of therapeutic agents.



Scheme 4 Schematic illustration of the experimental set-up for EHD processing using a triple-needle device to fabricate tri-layer particles[62]

2.3 Chemical Approaches

(a) Solvent evaporation technique

Recently, Lee et al. showed how triple-layered polymeric particles can be fabricated through a simple, economical, reliable, and versatile one-step emulsion solvent evaporation technique.[63] Key fabrication parameters that affect the formation of these triple-layered polymeric microparticles comprising poly(DL-lactide-co-glycolide) (50:50), poly(L-lactide), and poly(ethylene-co-vinyl acetate) (40% vinyl acetate) are discussed along with their formation mechanisms. Layer thickness and the configurations of these microparticles are altered by changing the polymer mass ratios. Concurrently, it was shown that drugs can be localized in specific layers of the particles. This fabrication process can therefore be used to tailor microparticle designs, thus allowing such "designer" particulate drug-delivery systems to function across a wide range of applications.

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(b) Near-critical micellization (NCM) method

It is highly desirable to develop a translatable drug-loading process that uses FDA approved building blocks to fabricate drug-loaded micelles with minimized burst release, consequently mitigating side effects while enhancing therapeutic efficacy. In this regard, scientists have developed a near-critical micellization (NCM) method and fabricated PEG−PCL micelles loaded with paclitaxel.[64] The resulting micelles had much higher drug loading but did little to reduce burst release. It was subsequently hypothesized that adding a protective layer on the drug containing core would reduce any burst-release. In principle, this can be accomplished with a suitable multiblock (at least triblock) copolymer via a precisely controlled sequential block collapse, while carefully synchronizing with drug nucleation and encapsulation. Although this is almost impossible with the conventional liquid-solvent−antisolvent micellization method, it is attainable with the NCM method. This is because the NCM method relies on compressed, near-critical gases that allow for precise, pressure-tuned control of each crucial structure forming stage, as illustrated in **Figure 3**.

Middleton et al. proved this hypothesis using ABC-type triblock copolymers with poly(ethylene glycol) (PEG) as the hydrophilic, corona-forming block on one end, poly(ε-caprolactone) (PCL) as the hydrophobic, core-forming block on the other end, and a middle block that should form a protective "shell" around the core. For this auxiliary middle block, they have selected two models: semi-crystalline poly(L-lactide) (PLLA), and amorphous poly(D,L-lactide) (PDLLA).[65] A structurally analogous reference diblock is PEG-b-(PDLLA-co-PCL), with a hydrophobic segment made of randomly distributed D,L-lactide and ε-caprolactone monomers. This system is demonstrated to produce benign, stable nanoparticles made of PEG-b-PLLA-b-PCL triblock copolymers that are not only solvent-free but also paclitaxel-rich. Ultimately, the ability to control the near-critical fluid phase transitions of the self-organizing block copolymers using pressure opens the door for a simple, yet precise, design and preparation of drug and gene delivery vehicles.

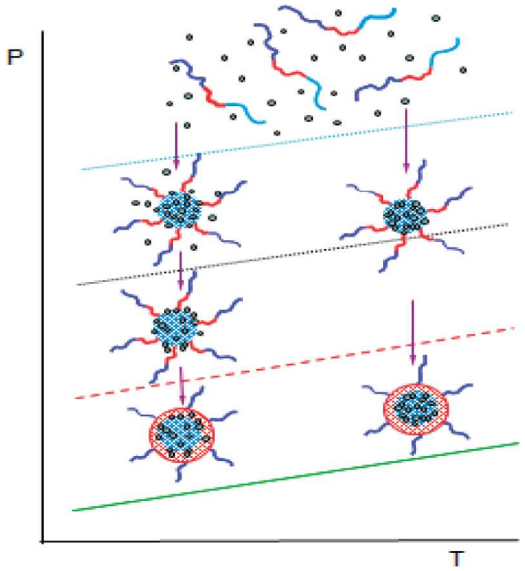

Figure 3 Sequence of block collapse and drug encapsulation upon near-critical micellization[65]

3. Particle Core / Templates

A diverse set of (i) templates, (ii) coating materials, and (iii) methods has to be chosen in fabricating multifunctional, multilayered particles. After layering onto a template, the sacrificial component is sometimes dissolved to form hollow shell particles; otherwise it remains as the core of core−shell particles, whereby it functions as a reservoir for drugs or bioimaging agents. Generally, templates can be made of organic (soft) or inorganic (hard) materials, of which the latter can be further classified into porous or nonporous.[6] Silica, gold, zinc oxides are examples of nonporous hard templates. Silica particles can be dissolved using hydrofluoric acid in order to prepare hollow capsules. This method has been successfully used to create capsules loaded with therapeutics.[66] Another attractive nonporous candidate is gold nanoparticles. They provide additional imaging functionality because of their excellent contrast in electron microscopy and computerized tomography; likewise for quantum dots, which possess intrinsic fluorescence properties. Besides gold nanoparticles, iron oxide nanoparticles also received a great deal of attention, especially for bioimaging purposes.[67-69] Porous templates such as mesoporous silica and calcium carbonate particles are also popular choices for encapsulating compounds that requires a high loading capacity.[70-72] Soft templates, on the other hand, are generally polymeric-based, examples of which are liposomes, micelles, polymers. In essence, the choice of template together with the intended subsequent layers should be carefully considered to yield the desired outcome, because the template governs the overall size and shape of the final particle, as well as its internal structure and the way therapeutic compounds are loaded.

3.1 Hard templates: Nonporous

(a) Upconversion Nanoparticles (UCNPs)

Lanthanide-doped rare-earth UCNPs, which are able to emit high-energy photons under excitation by near-infrared (NIR) light, have found potential applications in many different fields including nano-biomedicine.[73] Upconversion luminescence (UCL) imaging based on UCNPs shows a number of unique advantages over traditional fluorescence imaging, such as enhanced tissue penetration, better photostability, while eliminating auto fluorescence background. This allows for ultrasensitive *in vivo* detection.[73] UCNPs have also shown great promise in cancer therapies including their use as drug delivery carriers to enable NIR-induced photodynamic therapy, and to realize imaging guided cancer theranostics.[74, 75] Lately, two groups reported the use of UCNPs, as the core, for NIR-triggered gene delivery.[76, 77]

He et al. designed a new type of surface-coating strategy to functionalize Gd^{3+}-containing UCNPs, rendering them with excellent gene transfection capability.[78] In this design, UCNPs that serve as dual-modal imaging probes for optical imaging and MRI, are first conjugated with PEG to acquire physiological stability before coating with one or two layers of PEI polymer to achieve gene loading ability (**Figure 4**). They reported that two layers of PEI coating offered reduced cytotoxicity with enhanced gene transfection compared to those with only one layer of PEI. This work not only highlights the promise of UCNP-PEG-2PEI as a novel imaging-trackable nanovector for safe and efficient gene delivery, but also suggests that well-engineered surface chemistry is critical for the development of other types of gene-delivery nano-vectors.

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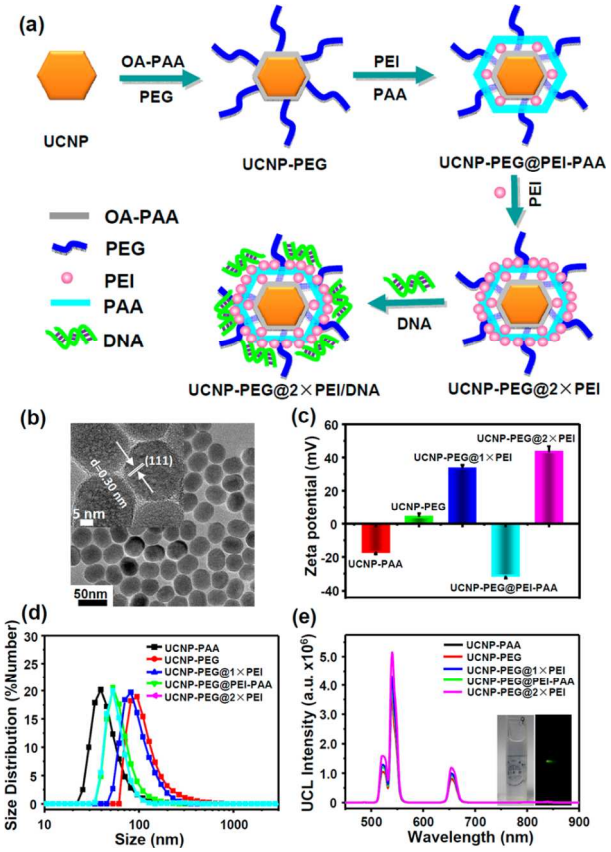

Figure 4. Preparation of UCNP-based gene vector. (a) Schematic illustration showing the synthesis of UCNP-PEG-2PEI and the subsequent pDNA binding. (b) TEM images of as-made UCNPs. The inset shows the HRTEM image of UCNPs. (c) ζ potentials (d) and DLS data of UCNPs after various layers of coatings. (e) UCL spectra after various layers of coatings. Inset shows UCNP-PEG-2PEI sample under ambient light (left) or exposed to a 980 nm laser (right).[78]

(b) Gold nanoparticles

In order to fabricate enzyme-assisted siRNA delivery system, Lee et al.[79] selected gold nanoparticles (AuNPs) as the core material for this purpose. AuNPs were chosen for their unique properties, including uniform size, shape-dependent optical and electronic features, biocompatibility, and feasibility for surface modification.[80] They devised a simple LbL assembly approach to prepare a new low toxicity enzyme-assisted siRNA delivery system (**Figure 5**).[79] It is evident that high loading of siRNA can be achieved by coating multiple layers of siRNA onto a nanoparticle and the enzyme-assisted release of siRNA can be controlled by the number of layers and degradability of the positively charged polymers. In addition, the shielding layers could protect the siRNA from degradation. A polypeptide in poly-l-lysine (PLL), which has previously used as a gene-delivery vector and drug carrier, was selected as the positively charged polyelectrolyte for its protease degradability.[81] PLL was cleaved by lysosomal cathepsin B *in vivo*, which is often up-regulated in cancer cells and inflamed cells, resulting in a bright fluorescent signal in the area with high enzymatic activity.[82, 83] Similarly, the progressive degradation of PLL inside of the cell is expected to trigger a slow release of siRNA, resulting in a prolonged gene-silencing effect. To achieve this, they have successfully fabricated densely packed multilayered sRAuNPs by layering oppositely charged PLL and siRNA on the surface of AuNPs. These multilayered sRAuNPs could deliver siRNA into tumor cells and silence its target gene effectively. A persistent siRNA inhibition effect was achieved as a result of incorporated protease-assisted slow-release design.

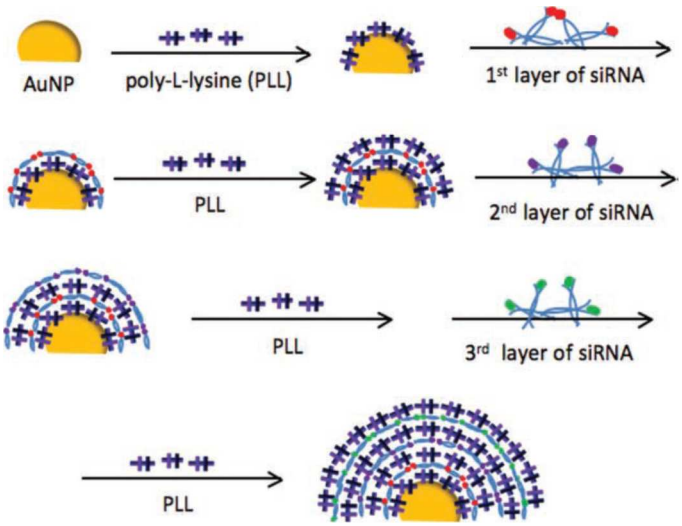

Figure 5. Preparation of multilayer siRNA-coated AuNPs (sRAuNPs) using siRNA and PLL as the charged polyelectrolytes.[79]

In another report, Reum et al. demonstrated a novel drug delivery system based on LbL technique, using AuNPs as a template for the delivery of water-insoluble anticancer drugs.[84] Very recently, Oraevsky et al.[85] developed dual contrast agents or nanocomposite particles that are designed to enhance optoacoustic-ultrasonic imaging. The contrast agents or particles have a core (i.e. gold nanorod) designed to enhance response to incident transient ultrasonic pressure waves with at least two layers disposed around the core. The inner first layer is designed to effectively absorb incident transient optical waves, convert the absorbed optical energy into heat before conversion into acoustic pressure. The outer second layer thermally insulates the inner layer from the surrounding aqueous environment and enhances the generation of transient ultrasonic pressure waves during optoacoustic-ultrasonic imaging and sensing.

3.2 Hard templates: Porous

(a) Mesoporous silica nanoparticles (MSNs)

Mesoporous silica nanoparticles (MSNs) have drawn much attention as attractive candidates for drug delivery and biosensors[86-88], owing to their unique characteristics, such as high surface area-to-volume ratio that allows for high drug adsorption, stability against bioerosions, sustained release profile, good thermal stability, etc.[89] It is also well demonstrated that MSNs can be degraded in simulated body fluid and is biocompatible[90, 91]. Recently, amino PGOHMA/PAA LbL-coated silica nanoparticles showed promising results as doxorubicin hydrochloride (DOX) carriers[92]. Amino poly(glycerol methacrylate)s (PGOHMAs) and poly(acrylic acid) (PAA) are reactive, inexpensive, hydrophilic, biocompatible and pH-responsive[93], making them attractive for drug delivery. Carboxylated MSNs (MSN-COOH), aminated MSN (MSN-NH_2) and H-MSN, were used as templates for MSNPEN (MSN-polyelectrolytes nanoparticles) assembly. These MSNPENs can be used as reservoirs for drug storage and protection. In a recent study led by Sun et al., the anti-cancer drug DOX was pre-loaded into the mesoporous interior of H-MSN, and subsequently stepwise-coated with amino-PGOHMAs/PAA via LbL assembly to obtain MSNPENs.[94]

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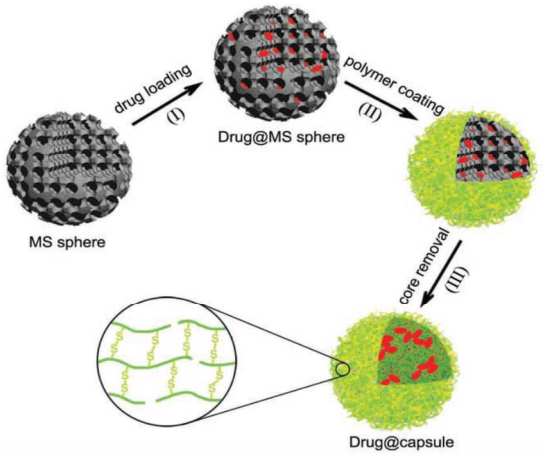
$MSNPEN_1$, with MSN-COOH core, holds more DOX than $MSNPEN_2$, with MSN-NH_2 core. The loading condition of $MSNPEN_1$ was optimized to be at pH 8.0 with four layers of polyelectrolytes coatings. They have also observed that nano-assemblies constructed from star-shaped polymer could encapsulate DOX with higher efficiency than its linear counterpart. The cumulative release of DOX from $MSNPEN_1$ showed a low leakage at pH 7.4, while significantly enhanced to 60% at pH 5.0 and over 70% at pH 2.0. These results demonstrated that these composite nanoparticles were pH responsive, and can be potentially applied as targeted releasing drug nanocarriers for cancer therapy.

(b) Hollow mesoporous silica particles (HMS)

Hollow mesoporous silica (HMS) particles exhibit a much higher drug loading capacity compared to the conventional mesoporous silica materials due to their large hollow cavities for drug storage.[95-97] Wang et al. demonstrated a facile and versatile approach for encapsulating water-insoluble compounds (e.g. thiocoraline, paclitaxel) in polymer capsules through mesoporous silica particle-mediated drug loading and subsequent generation of a polymer multilayer shell using the LbL technique (**Scheme 5**).[98] After removal of the silica particles, the water-insoluble small compounds agglomerate into clusters, and are retained in the polymer capsules. This method yields nanocapsules with a high degree of drug loading, retained drug activity, and avoids the need for drug stabilization with a lipophilic phase or surfactant, or within smaller carriers. These drug-loaded capsules have similar cytotoxicity to the free drug towards colorectal cancer cells.



Scheme 5. Schematic illustration of the encapsulation of water-insoluble therapeutics in PMA SH nanocapsules via mesoporous silica templating. (I) Loading of water-insoluble therapeutics into MS particles; (II) LbL assembly of a multilayer polymer shell on the surface of the drug-loaded MS particles; (III) Removal of the MS particle, leading to polymer capsule encapsulated therapeutics.[98]

4. Particle Shell / Responsive Layers

The design of smart polymeric carriers with tunable properties is of considerable interest for applications in drug delivery. Prerequisites for successful carriers are biocompatibility, stability in the bloodstream, low-fouling characteristics, high encapsulation efficiencies as well as the ability to release encapsulated cargo on demand. The triggered release of payload has been the subject of extensive research during the last decade.[99, 100] Various mechanisms and stimuli have been reported to induce the release of encapsulated cargo either by applying an external stimulus (e.g., temperature) or a biological trigger (e.g., pH, redox potential, or enzymatic change). Carriers that are responsive to biological changes are particularly desirable as they can, for example, innately respond upon sensing extra/intracellular changes. Generally, biological triggers induce a change in the permeability of the carrier systems leading to a response.

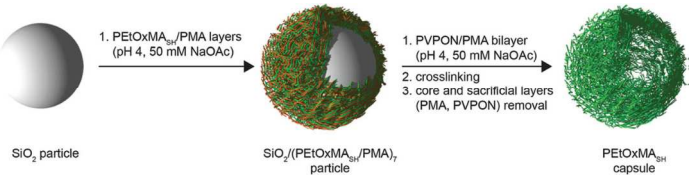
4.1 Single responsive layers

(a) Redox responsive layers

H-bonded assembled films are of particular interest because they can be designed to be responsive under biologically relevant conditions. Unlike electrostatically assembled films, they are not permanently charged, thus generally endowing them with lower fouling properties. Low-fouling coating decrease or resist nonspecific protein adsorption, which is a crucial property for advanced polymer carriers because they can allow efficient cargo delivery to a targeted site. Kempe et al. recently introduced poly(2-oxazoline)s (POxs) for the assembly of low-fouling polymer capsules.[101] It was shown that capsules prepared from brush-like POx exhibit low-fouling properties. In addition, the modularity of the cationic ring-opening polymerization (CROP) of functional 2-oxazolines provides access to highly functional polymer systems with tailorable properties. Polymers functionalized with redox-responsive groups have been used to prepare carriers that are responsive to biological triggers.[102, 103] For example, this can be realized by using disulfide and diselenide chemistry that exploit differences between intra- and extra-cellular redox potentials. Disulfide-stabilized systems are cleaved within cells due to the presence of glutathione (GSH) – an abundant intracellular thiol species.[101]

In another study by Kempe et al., synthesis of brush-like poly(2-ethyl-2-oxazoline) (PEtOx) with pendant thiol moieties and the fabrication of redox-responsive LbL-assembled PEtOx capsules were reported (**Scheme 6**).[104] These capsules are both intracellularly degradable and are comparatively lower fouling than the counterpart PMA SH (thiol functionalized poly(methacrylic acid) and PDPA Alk (alkyne containing poly(2-diisopropylaminoethyl methacrylate)) systems. The thiol/disulfide exchange reaction was employed for stabilization of the polymer films, and also endowed the films with redox responsive properties. Stabilization of these multilayers was achieved by disulfide formation, which renders the respective capsules degradable under reducing conditions both in a simulated biological environment (GSH) and intracellularly. The intracellular degradation of the PEtOx capsules in combination with the low-fouling properties makes them suitable for delivery of therapeutics.

Another report by Xia et al.,[105] where FITC-dextran-encapsulated hollow microcapsules were prepared via LBL assembly of a blend of biocleavable polycation (disulfide-containing) and non-biocleavable polycations (polymer structures[105]) and poly(sodium 4-styrenesulfonate) (PSS). The disulfide-containing microcapsules were cleaved when incubated under reductive conditions. The release of encapsulated FITC-dextran is reduction-sensitive, and the release rate of biomacromolecules is dependent on the w/w ratio of biocleavable polycation to nonbiocleavable polycation.



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Scheme 6 Preparation of disulfide-stabilized PEtOxMA SH capsules via hydrogen-bonded LbL assembly of PEtOxMA SH and PMA.[104]

(b) Glucose responsive layers

In the human body system, other than salt and pH, glucose concentration is also a very important factor. The concentration of glucose varies in different parts of the human body. For a healthy person the average extra-cellular (blood) concentration of glucose is ~5 mM[106]. On the other hand, cancer cells metabolize differently than normal cells and accumulate glucose faster than normal cells. In cancer cells, researchers believe that glucose concentration has a strong influence on the tumor hexokinase type II promoter. Maximal activity of this promoter has been found at 25 mM concentration[107]. Thus, consumption of glucose increases in cancer cell and this high glucose concentration can be utilized for targeting cancer cells.

Levy et al.[108] introduced a path to form glucose sensing multilayer thin film, and this assembly is suitable for sensing different carbohydrates as this membrane disintegrates rapidly in presence of glucose molecules even at very low concentration. In 2006, Geest et al.[109] introduced another glucose response multilayer assembly based on LbL electrostatic adsorption of a polyanion (PSS) and a phenylboronic acid containing polycation. In the presence of glucose, this multilayer assembly gets over-charged due to induction of negative charge on the boron atom that leads to a rapid disassembly of the whole membrane within five minutes. These multilayer assemblies are more applicable for fast release applications rather than controlled release applications. Manna et al.[110] have reported on the effect of glucose concentration on borate mediated LbL self-assembly of neutral PVA polymer and chitosan and also studied the disintegration of this material in presence of glucose molecules. Because of the presence of physically cross-linked PVA hydrogel inside the multilayer, the capsules morphology and size can be tuned. PVA-borate complex undergoes a strong interaction with glucose molecules and destroys the physically crosslinked PVA-borate complex. Based on this principle, they have developed a glucose-triggered releasing system. Disintegration of this multilayer membrane is evidenced with Confocal Laser Scanning Microscopy at higher concentration of glucose. The presence of borate in the multilayer wall provides the possibility for controlled release of anticancer drugs doxorubicin (encapsulated in PVA-borate/chitosan multilayer) by means of variable glucose concentration (**Figure 6**) – a smart anticancer drug delivery system.

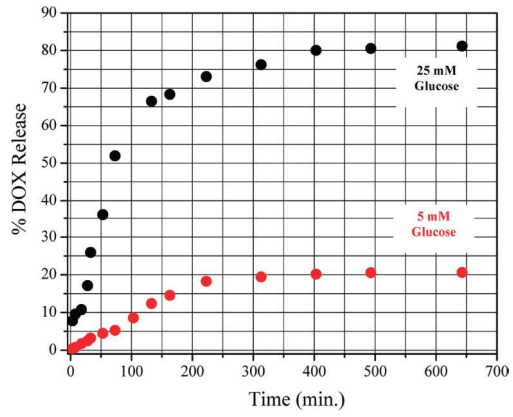

Figure 6 Glucose response DOX release from multilayer assembly[110]

4.2 Dual responsive layers

(a) Magnetic with thermal responsive polymer layers

Recent strategy is to incorporate smart polymer such as thermosensitive poly(N-isopropylacrylamide) (PNIPAAm) with magnetic cores to provide a temperature-sensitive drug release mechanism. These nanoparticles can be loaded with a hydrophilic drug and guided to the treatment site by an external magnetic field. Then, an external electromagnetic device can be used to locally raise the temperature above the polymer's lower critical solution temperature (LCST); consequently, the polymer structure collapses and releases the drug. The research carried out by Koppolu et al. developed dynamic nanoparticles capable of providing a dual drug-delivery mechanism. To achieve this, multilayered nanoparticles (MLNPs) with a magnetic core and two shells made up of temperature-sensitive polymers (PNIPAAm) and biodegradable polymers (PLGA) were synthesized (**Figure 7**).[111] PNIPAAm was immobilized onto the magnetic nanoparticles using a coupled silane agent and free radical polymerization of the N-isopropylacrylamide monomer. The resultant PNIPAAm magnetic nanoparticles were then encapsulated with PLGA by a double emulsion solvent evaporation technique using poly(vinyl alcohol) (PVA) as a surfactant.

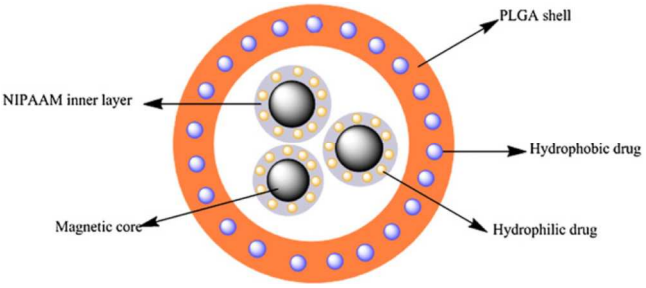

Figure 7 Drug encapsulated multilayered nano particles with a magnetic core and two shells made up of temperature-sensitive polymers (PNIPAAm) and biodegradable PLGA polymers.[111]

(b) pH and temperature responsive layers

In addition to temperature responsive layers, another popular choice is pH responsiveness. Particles are provided with a multilayer polymer coating of specific structure which ensures the gradual release of the active agent in desired range of pH. [112] Xu et al. utilized star polyelectrolytes (poly(N,N-dimethylaminoethyl methacrylate) (PDMAEMA)) with dual (temperature and pH) responsive properties to fabricate multiresponsive microcapsules via LbL assembly.[113] Ionic strength in the polyelectrolyte solution during the microcapsule assembly process has a significant influence on the thickness and permeability of microcapsules. With increasing pH, the permeability of microcapsules decreases, and the transition from "open" to "close" state for target molecules can be achieved within a narrow pH range (from pH 7 to 8). On the other hand, the overall size and permeability of the microcapsules decrease with increasing temperature, thus allowing reversibly loading and unloading the microcapsules with high efficiency. The organization and interaction of star polyelectrolytes within confined multilayer structure are the main driving forces for the responsiveness to external stimuli. The multiresponsive LbL microcapsules represent a novel category of smart microstructures as compared to traditional LbL microcapsules with "one-dimensional" response to a single stimulus. They also have the potential to mimic the complex responsive microstructures found in nature and find applications in drug delivery, smart coatings, microreactors, and biosensors.

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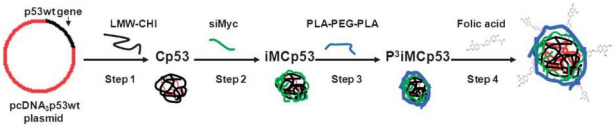
5. Therapeutic Formulations / Applications

5.1 Targeted delivery

Gene therapy is an elegant alternative to chemotherapy, without the severe side-effects associated with the latter. While viral gene delivery bears the risk of infection, polymer-aided delivery system suffers from low transfection efficacy combined with a relatively high cytotoxicity. Mandal et al. introduced a targeted small polymeric multicomponent particles (**Scheme 7**) that deliver siRNA and a plasmid simultaneously, correcting mutations in both cell growth and cell death.[114] In order to create a charged core on which LbL technique can be applied, the plasmid that encodes for the wild-type p53 in the polyanion was condensed onto a positively-charged low molecular weight chitosan (LMW-CHI) core. Subsequently, the positive charge of the condensed plasmid [Cp53] MCP acts as a base for the electrostatic binding of the negative siRNA. To further protect the polymeric particles from immune recognition, PEG in the form of neutral PLA-PEG-PLA was employed through H-bonding. These 300nm multilayered particles arrested cell proliferation while transfecting cancer cells with a wild-type p53 plasmid to induce apoptosis. A high selectivity along with a comparably high transfection efficacy was achieved through surface functionalizing with folic acid. As a result a transfection efficacy and gene expression of around 80–90% was achieved *in vitro* in different cancer cell lines holding promises for future gene therapies.



Scheme 7 Scheme of layer-wise construction of [Fo-P3iMCp53] MCPs.[114]

5.2 Triggered release

Zhu et al. proposed a concept to design an enzyme-triggered drug and gene co-delivery system through HMS/PLL (hollow mesoporous silica / poly(L-lysine)) particles, driven by electrostatic interaction between negatively-charged gene and positively-charged PLL on drug-loaded HMS particles (**Figure 8**).[115] While HMS particles provide high drug loading capacity, the coating with the gene/PLL layer by LbL caps the mesopores thus loading the gene. This co-delivery system provides an enzyme-triggered controlled release of drug and gene simultaneously, in which release rates are controlled by enzyme concentrations. This multilayered system has the advantage of enzyme-triggered controlled release of drug and gene, a promising strategy for therapeutic applications.

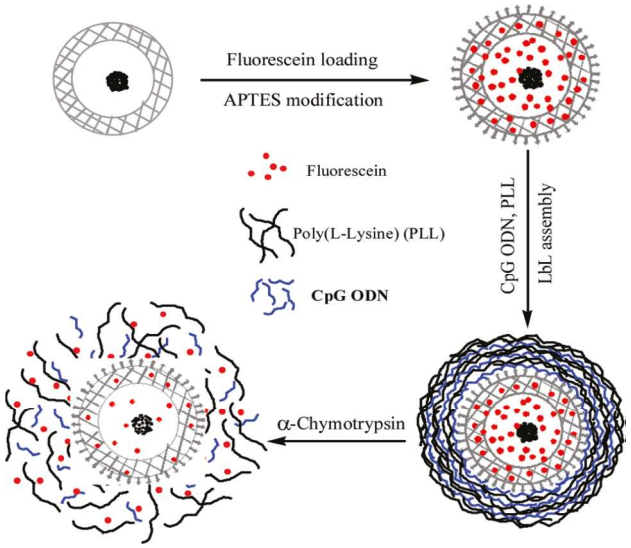


Figure 8 Schematic procedures for preparation of the fluorescein and CpG ODN-loaded HMS/PLL particles and enzyme-triggered release.[115]

5.3 Sustained release

Clinical trials using islets encapsulated in alginate microcapsules have shown some promise as a treatment for Type 1 diabetes. However, large numbers of islets are required for efficacy because of poor survival following transplantation. Encapsulation of islets in a permselective biomaterial may avoid the need for immunosuppressive drugs to prevent transplant rejection. The ability to encapsulate islets in multilayered microcapsules in which an angiogenic protein is released from the outer layer may enhance the viability of transplanted islets, thus reducing the number of islets required for treatment. To achieve this goal, Brey et al. designed a biocompatible, multilayered alginate microcapsules with a permselective poly-L-ornithine (PLO) membrane that can be used for the dual purpose of encapsulating cells in the inner core, while sustaining the release of angiogenic proteins from the outer layer (**Figure 9**).[116]

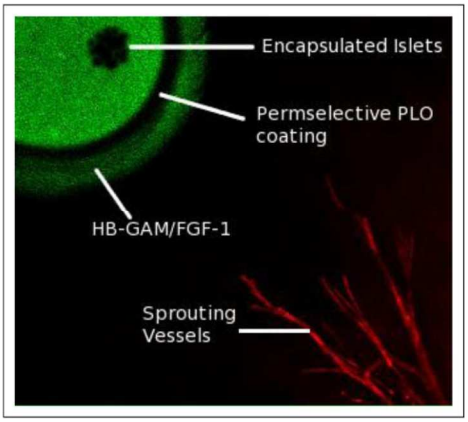


Figure 9 A visual schematic of the concept behind multilayered alginate microbeads. Islets are encapsulated in the inner core and an angiogenic protein (HB-GAM/FGF-1) in the outer alginate layer. The permselective PLO membrane prevents the diffusion of large molecules such as antibodies but allows for the exchange of solutes such as glucose, oxygen, and insulin. When implanted, the protein is

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released from the outer layer and stimulates neovascularization toward the bead.[116]

The incorporation of low molecular weight drugs and therapeutic peptides into multilayered films assembled via LbL can potentially provide a means to target small molecules, while tuning their release. Gras et al. described the use of both hydrophobic and electrostatic interactions to incorporate a tridecapeptide anti-inflammatory hormone, i.e. α-melanocyte stimulating hormone (α-MSH), as a building block onto a multilayered assembly of hyaluronic acid (HA) and chitosan (CS) on poly(lactic-glycolic acid) (PLGA) surfaces.[117] A range of switching layers, including a neutral lipid, dioleylphosphatidylcholine (DOPC), a negatively charged lipid mixture DOPC/dioleylphosphatidylserine (DOPS) and a negatively-charged polysaccharide in HA, were investigated for their ability to support subsequent HA and CS layers. This assembly was shown to be stable at physiological pH and was successfully applied to particulate systems.

Tissue regeneration may be stimulated by growth factors (GFs), but to be effective, delivery must be sustained from scaffolds to overcome the short half-life of these biomolecules. One promising approach is to couple growth factors onto biomaterial surfaces to make them readily bioavailable. LbL was used to construct a multilayered polyelectrolyte delivery system on the surface of poly(lactic-co-glycolic) acid constructs (**Figure 10**).[118] Here, surfaces of hydrophobic PLGA microspheres were aminolyzed to create a hydrophilic surface with functional groups on which multilayers of HA, CS, Hep, and bFGF were constructed by LbL. Crosslinked capping multilayers provided a more tortuous diffusion path that prevents burst release and achieves sustained release (**Figure 11**). This delivery system is biodegradable, and Hep incorporated within the assembly preserves bFGF activity, providing an advantage over the delivery of free bFGF that would otherwise have a short half-life. LbL technique offers great flexibility as the number of Hep-bFGF layers can be increased to boost bFGF loading. Layers of CS and HA can also be added or subtracted to control the rate of bFGF release. This delivery system can also deliver single or multiple combinations of GFs that bind to Hep or other charged biomolecules. A variety of polyelectrolytes can also be employed to create a range of tailorable multilayered microspheres to enhance tissue regeneration, particularly in soft tissue. These microspheres could also be used in combination with a biodegradable scaffold, where mechanical support is required.

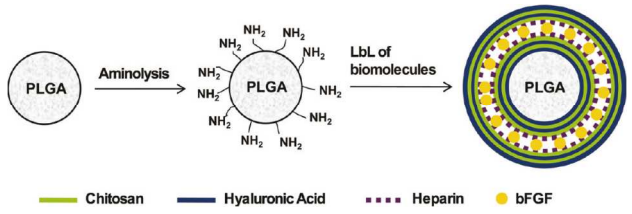


Figure 10 Schematic of the build-up of a multilayered structure on the surface of PLGA microspheres. Hyaluronic acid (HA) was added first on the surface of the aminolyzed microspheres; this was followed by chitosan (CS). bFGF was at the centre of the assembly and was protected by an upper and lower heparin (Hep) layer (not to scale).[118]

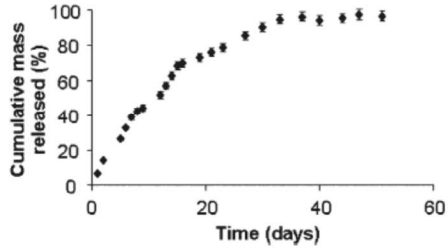
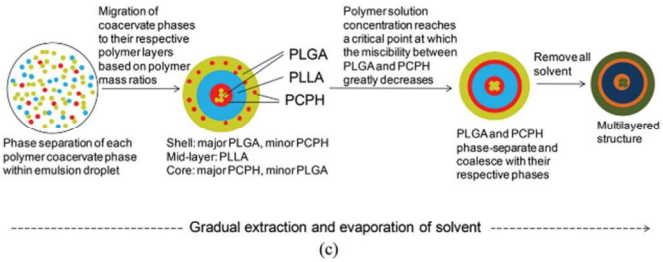


Figure 11 Release of bFGF from layer-by-layer coated PLGA microspheres as assessed by ELISA. The cumulative mass of bFGF released is expressed as a percentage of the mass of bFGF loaded onto the PLGA microspheres.[118]

5.4 Dual drug delivery: controlled release of two drugs

In chemotherapy, repeated dosing of a combination of drugs is usually given over a prolonged period. While single-layered particles have been widely investigated for delivering chemotherapeutic drugs, these conventional particles have several inherent limitations, including burst release, the inability to provide a zero-order, time-delayed or pulsatile release of therapeutic agents. Multilayered particles are shown to have the potential to overcome these issues.[119-123] Recently, Lee et al. developed an attractive strategy to fabricate dual-drug-loaded, multilayered microparticles and investigated their antitumor efficacy compared with single-drug-loaded particles. The scheme of the formation of a multilayered PLGA/PLLA/PCPH particle from an emulsion droplet is shown below (**Scheme** 8).[8]



Scheme 8 Schematic illustration (not to scale) of the proposed mechanism involved in the formation of the PLGA/PLLA/PCPH microparticles.[8]

The results showed that hydrophilic DOX and hydrophobic paclitaxel (PTX) are localized in the PLGA shell and in the poly(L-lactic acid) (PLLA) core, respectively. By introducing another polymer in poly [(1,6-bis-carboxyphenoxy) hexane] (PCPH) to PLGA/PLLA microparticles, PTX was found to be localized in the PLLA and PCPH mid-layers, whereas DOX was found in the PLGA shell and core. PLGA/PLLA/PCPH multilayered microparticles with denser shells allowed for better controlled release of DOX. A delayed release of PTX was also observed with the addition of PCPH. Controlled co-delivery of DOX and PTX from multilayered microparticles to three dimensional MCF-7 spheroid demonstrated a greater reduction in spheroid growth rate as compared to single-drug-loaded particles. This study provides mechanistic insights into how distinctive structure of multilayered microparticles can be designed to modulate the release profiles of anticancer drugs, and how co-delivery of drugs can potentially provide better antitumor responses. Briefly, the potential applications of multilayered particles can be summarized through **Scheme 9**.

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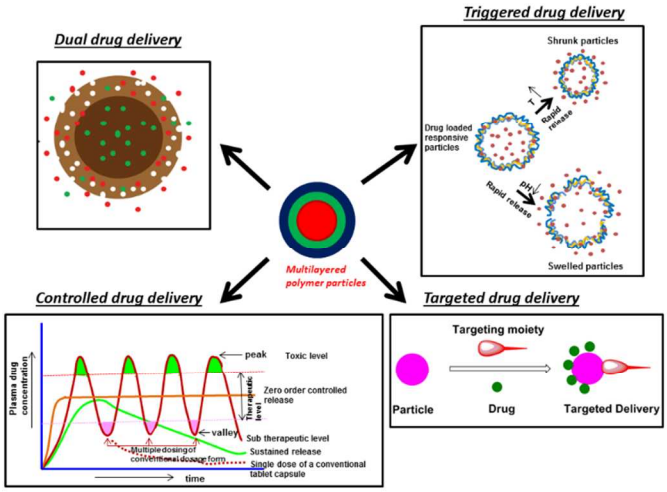

Scheme 9 Schematic illustration of the potential therapeutic applications of multilayer particles.

6. Conclusions & Outlook

In recent years, research in multilayered polymer particles have made rapid progresses. This includes the understanding of theoretical principles that underlies its fabrication process, and using this to develop a commercializable pharmaceutical product. These highly versatile multilayered particles, that enable cargo encapsulation, controlled release, and surface functionalization, offer a range of unique opportunities as tailor-made delivery vehicles to enhance the efficacy and specificity of therapeutics. Many of these proof-of-concept studies were reviewed here. However, significant challenges still remain that hinder their full potential as novel therapeutics. Here is a list of issues (with partial solution) that have to be resolved in order to translate this technology from bench-to-bedside:

- Finding an efficient fabrication technique that allows for scale up production. This problem can be partly resolved by adopting immersive polymer assembly technique as discussed in section 2.2b.
- Achieving size smaller than 200 nm using multilayer technologies is certainly a problem, except for the 'Near critical micellization' method (section 2.3b). But, when particles are used for controlled release in a drug 'depot', they need not to be less than 200 nm. They can work as sustained release carrier for long term delivery purposes.
- Resolving aggregation issues, particularly of nanoparticles. This also can be partially avoided by incorporating polymers that can provide electro-steric stabilization at physiological pH.
- Enhance reproducibility of results, especially pertaining to release kinetics.
- Understand the detailed mechanism of shell permeation, controlled delivery and of the structure of the core.
- Understand its interaction with biological systems, such as cells and tumours.
- Study its long-term stability while in storage or in use.
- Establish the reliability and efficacy of multilayered delivery systems in animal and clinical trials.

Addressing these issues will be the main challenges to realize the commercialization of these multilayered delivery systems as therapeutic formulations of the future.

Acknowledgements

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References

1. Y. Yan, G. K. Such, A. P. R. Johnston, J. P. Best and F. Caruso, *ACS Nano*, 2012, 6, 3663-3669.
2. G. Decher, J. D. Hong and J. Schmitt, *Thin Solid Films*, 1992, 210–211, Part 2, 831-835.
3. G. Decher, *Science (Washington, D. C.)*, 1997, 277, 1232-1237.
4. F. Caruso, R. A. Caruso and H. Moehwald, *Science (Washington, D. C.)*, 1998, 282, 1111-1114.
5. E. Donath, G. B. Sukhorukov, F. Caruso, S. A. Davis and H. Möhwald, *Angewandte Chemie International Edition*, 1998, 37, 2201-2205.
6. Y. Wang, A. S. Angelatos and F. Caruso, *Chem. Mater.*, 2008, 20, 848-858.
7. Y. Yan, G. K. Such, A. P. R. Johnston, H. Lomas and F. Caruso, *ACS Nano*, 2011, 5, 4252-4257.
8. W. L. Lee, W. M. Guo, V. H. B. Ho, A. Saha, H. C. Chong, N. S. Tan, E. Widjaja, E. Y. Tan and S. C. J. Loo, *Small*, 2014, 10, 3986-3996.
9. Z. Poon, D. Chang, X. Zhao and P. T. Hammond, *ACS Nano*, 2011, 5, 4284-4292.
10. W. Tong, X. Song and C. Gao, *Chemical Society Reviews*, 2012, 41, 6103-6124.
11. G. K. Such, A. P. R. Johnston and F. Caruso, *Chemical Society Reviews*, 2011, 40, 19-29.
12. S. De Koker, R. Hoogenboom and B. G. De Geest, *Chemical Society Reviews*, 2012, 41, 2867-2884.
13. T. Serizawa, S. Kamimura, N. Kawanishi and M. Akashi, *Langmuir*, 2002, 18, 8381-8385.
14. Y. Zhang, S. Yang, Y. Guan, W. Cao and J. Xu, *Macromolecules*, 2003, 36, 4238-4240.
15. A. P. R. Johnston, E. S. Read and F. Caruso, *Nano Letters*, 2005, 5, 953-956.
16. T. Kida, M. Mouri and M. Akashi, *Angewandte Chemie International Edition*, 2006, 45, 7534-7536.
17. Z. Wang, Z. Feng and C. Gao, *Chemistry of Materials*, 2008, 20, 4194-4199.
18. X. Shi, S. Wang, X. Chen, S. Meshinchi and J. R. Baker, *Molecular Pharmaceutics*, 2006, 3, 144-151.
19. S. H. Wang, X. Shi, X. Chen and J. R. Baker, *Macromolecular Bioscience*, 2009, 9, 429-436.
20. N. Kato, P. Schuetz, A. Fery and F. Caruso, *Macromolecules*, 2002, 35, 9780-9787.
21. J. A. Hiller, J. D. Mendelsohn and M. F. Rubner, *Nat Mater*, 2002, 1, 59-63.
22. T. C. Wang, R. E. Cohen and M. F. Rubner, *Advanced Materials*, 2002, 14, 1534-1537.
23. J. A. Hiller and M. F. Rubner, *Macromolecules*, 2003, 36, 4078-4083.
24. X. Shi, M. Shen and H. Möhwald, *Progress in Polymer Science*, 2004, 29, 987-1019.
25. P. Schuetz and F. Caruso, *Advanced Functional Materials*, 2003, 13, 929-937.

Journal of Materials Chemistry B Accepted Manuscript

26. S. Y. Yang, J. D. Mendelsohn and M. F. Rubner, *Biomacromolecules*, 2003, 4, 987-994.
27. J. D. Mendelsohn, S. Y. Yang, J. A. Hiller, A. I. Hochbaum and M. F. Rubner, *Biomacromolecules*, 2003, 4, 96-106.
28. S. E. Burke and C. J. Barrett, *Langmuir*, 2003, 19, 3297-3303.
29. S. A. Sukhishvili, V. Kozlovskaya and E. Kharlampieva, Google Patents, 2005.
30. Y. Zhang, Y. Guan, S. Yang, J. Xu and C. C. Han, *Advanced Materials*, 2003, 15, 832-835.
31. J. Chen, V. Kozlovskaya, A. Goins, J. Campos-Gomez, M. Saeed and E. Kharlampieva, *Biomacromolecules*, 2013, 14, 3830-3841.
32. I. Erel-Unal and S. A. Sukhishvili, *Macromolecules*, 2008, 41, 3962-3970.
33. I. Erel-Unal and S. A. Sukhishvili, *Macromolecules*, 2008, 41, 8737-8744.
34. V. Kozlovskaya, E. Kharlampieva, I. Drachuk, D. Cheng and V. V. Tsukruk, *Soft Matter*, 2010, 6, 3596-3608.
35. S. Y. Yang, D. Lee, R. E. Cohen and M. F. Rubner, *Langmuir*, 2004, 20, 5978-5981.
36. T. Serizawa, K. Nanameki, K. Yamamoto and M. Akashi, *Macromolecules*, 2002, 35, 2184-2189.
37. L. Richert, F. Boulmedais, P. Lavalle, J. Mutterer, E. Ferreux, G. Decher, P. Schaaf, J.-C. Voegel and C. Picart, *Biomacromolecules*, 2004, 5, 284-294.
38. V. Kozlovskaya, S. Ok, A. Sousa, M. Libera and S. A. Sukhishvili, *Macromolecules*, 2003, 36, 8590-8592.
39. B. de Boer, H. K. Simon, M. P. L. Werts, E. W. van der Vegte and G. Hadziioannou, *Macromolecules*, 1999, 33, 349-356.
40. R. K. O'Reilly, M. J. Joralemon, C. J. Hawker and K. L. Wooley, *Journal of Polymer Science Part A: Polymer Chemistry*, 2006, 44, 5203-5217.
41. M. Husemann, D. Mecerreyes, C. J. Hawker, J. L. Hedrick, R. Shah and N. L. Abbott, *Angewandte Chemie International Edition*, 1999, 38, 647-649.
42. O. Prucker, M. Schimmel, G. Tovar, W. Knoll and J. Rühe, *Advanced Materials*, 1998, 10, 1073-1077.
43. R. P. Quirk, R. T. Mathers, T. Cregger and M. D. Foster, *Macromolecules*, 2002, 35, 9964-9974.
44. B. Zhao and W. J. Brittain, *Macromolecules*, 2000, 33, 342-348.
45. X. Kong, T. Kawai, J. Abe and T. Iyoda, *Macromolecules*, 2001, 34, 1837-1844.
46. M. Husseman, E. E. Malmström, M. McNamara, M. Mate, D. Mecerreyes, D. G. Benoit, J. L. Hedrick, P. Mansky, E. Huang, T. P. Russell and C. J. Hawker, *Macromolecules*, 1999, 32, 1424-1431.
47. W. H. Yu, E. T. Kang and K. G. Neoh, *Langmuir*, 2005, 21, 450-456.
48. Q. Wang, L. Liu and W. Yang, *Polym. J*, 2008, 40, 192-197.
49. Q. Wang, L. Liu and W. Yang, *Polymer*, 2007, 48, 6581-6588.
50. G. Schneider and G. Decher, *Nano Letters*, 2004, 4, 1833-1839.
51. V. Saini, *RJC*, 2009, 2, 447-450.
52. E. S. Nuwayser, Google Patents, 2007.
53. W. L. Lee, J. W. M. Tan, C. N. Tan and S. C. J. Loo, *PLoS ONE*, 2014, 9, e114284.
54. A. Qi, P. Chan, J. Ho, A. Rajapaksa, J. Friend and L. Yeo, *ACS Nano*, 2011, 5, 9583-9591.
55. A. Voigt, H. Lichtenfeld, G. B. Sukhorukov, H. Zastrow, E. Donath, H. Bäumler and H. Möhwald, *Industrial & Engineering Chemistry Research*, 1999, 38, 4037-4043.
56. C. Priest, A. Quinn, A. Postma, A. N. Zelikin, J. Ralston and F. Caruso, *Lab on a Chip*, 2008, 8, 2182-2187.
57. C. Kantak, S. Beyer, L. Yobas, T. Bansal and D. Trau, *Lab on a Chip*, 2011, 11, 1030-1035.
58. J. J. Richardson, H. Ejima, S. L. Lörcher, K. Liang, P. Senn, J. Cui and F. Caruso, *Angewandte Chemie International Edition*, 2013, 52, 6455-6458.
59. J. J. Richardson, K. Liang, K. Kempe, H. Ejima, J. Cui and F. Caruso, *Adv Mater*, 2013, 25, 6874-6878.
60. S. T. Dubas and J. B. Schlenoff, *Macromolecules*, 1999, 32, 8153-8160.
61. S. T. Dubas and J. B. Schlenoff, *Langmuir*, 2001, 17, 7725-7727.
62. S. Labbaf, S. Deb, G. Cama, E. Stride and M. Edirisinghe, *Journal of Colloid and Interface Science*, 2013, 409, 245-254.
63. W. L. Lee, E. Widjaja and S. C. J. Loo, *Small*, 2010, 6, 1003-1011.
64. Z. L. Tyrrell, Y. Shen and M. Radosz, *The Journal of Physical Chemistry C*, 2011, 115, 11951-11956.
65. J. C. Middleton and A. J. Tipton, *Biomaterials*, 2000, 21, 2335-2346.
66. A. L. Becker, A. P. Johnston and F. Caruso, *Small*, 2010, 6, 1836-1852.
67. X. Shi, S. H. Wang, S. D. Swanson, S. Ge, Z. Cao, M. E. Van Antwerp, K. J. Landmark and J. R. Baker, *Advanced Materials*, 2008, 20, 1671-1678.
68. S. H. Wang, X. Shi, M. Van Antwerp, Z. Cao, S. D. Swanson, X. Bi and J. R. Baker, *Advanced Functional Materials*, 2007, 17, 3043-3050.
69. H. Cai, K. Li, M. Shen, S. Wen, Y. Luo, C. Peng, G. Zhang and X. Shi, *Journal of Materials Chemistry*, 2012, 22, 15110-15120.
70. A. Dong, Y. Wang, Y. Tang, N. Ren, Y. Zhang and Z. Gao, *Chemistry of Materials*, 2002, 14, 3217-3219.
71. G. B. Sukhorukov, D. V. Volodkin, A. M. Gunther, A. I. Petrov, D. B. Shenoy and H. Mohwald, *Journal of Materials Chemistry*, 2004, 14, 2073-2081.
72. Y. Wang, A. Yu and F. Caruso, *Angewandte Chemie International Edition*, 2005, 44, 2888-2892.
73. F. Wang and X. Liu, *Chemical Society Reviews*, 2009, 38, 976-989.
74. Y. Zhou, W. Chen, J. Zhu, W. Pei, C. Wang, L. Huang, C. Yao, Q. Yan, W. Huang, J. S. C. Loo and Q. Zhang, *Small*, 2014, 10, 4874-4885.
75. Y. Zhou, W. Chen, J. Zhu, W. Pei, C. Wang, L. Huang, C. Yao, Q. Yan, W. Huang, J. S. C. Loo and Q. Zhang, *Small*, 2014, 10, 4802-4802.
76. M. K. G. Jayakumar, N. M. Idris and Y. Zhang, *Proceedings of the National Academy of Sciences*, 2012, 109, 8483-8488.
77. Y. Yang, F. Liu, X. Liu and B. Xing, *Nanoscale*, 2013, 5, 231-238.
78. L. He, L. Feng, L. Cheng, Y. Liu, Z. Li, R. Peng, Y. Li, L. Guo and Z. Liu, *ACS Appl Mater Interfaces*, 2013, 5, 10381-10388.
79. S. K. Lee, M. S. Han, S. Asokan and C. H. Tung, *Small*, 2011, 7, 364-370.
80. M. C. Daniel and D. Astruc, *Chem Rev*, 2004, 104, 293-346.
81. L. C. Smith, J. Duguid, M. S. Wadhwa, M. J. Logan, C.-H. Tung, V. Edwards and J. T. Sparrow, *Advanced Drug Delivery Reviews*, 1998, 30, 115-131.
82. Y. Choi, R. Weissleder and C.-H. Tung, *Cancer Research*, 2006, 66, 7225-7229.
83. M. Funovics, R. Weissleder and C.-H. Tung, *Anal Bioanal Chem*, 2003, 377, 956-963.
84. N. Reum, C. Fink-Straube, T. Klein, R. W. Hartmann, C. M. Lehr and M. Schneider, *Langmuir*, 2010, 26, 16901-16908.
85. A. A. Oraevsky, A. Liopo and S. A. Ermilov, Google Patents, 2014.
86. M. Manzano and M. Vallet-Regi, *Journal of Materials Chemistry*, 2010, 20, 5593-5604.
87. Q. He, J. Shi, F. Chen, M. Zhu and L. Zhang, *Biomaterials*, 2010, 31, 3335-3346.
88. Q. He and J. Shi, *Journal of Materials Chemistry*, 2011, 21, 5845-5855.
89. J. L. Vivero-Escoto, I. I. Slowing, B. G. Trewyn and V. S. Y. Lin, *Small*, 2010, 6, 1952-1967.
90. Q. He, J. Shi, M. Zhu, Y. Chen and F. Chen, *Microporous and Mesoporous Materials*, 2010, 131, 314-320.
91. W. Zhai, C. He, L. Wu, Y. Zhou, H. Chen, J. Chang and H. Zhang, *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 2012, 100B, 1397-1403.
92. Y. Sun, H. Gao, Y.-W. Yang, A. Wang, G. Wu, Y. Wang, Y. Fan and J. Ma, *Journal of Biomedical Materials Research Part A*, 2013, 101A, 2164-2173.
93. H. Gao, X. Lu, Y. Ma, Y. Yang, J. Li, G. Wu, Y. Wang, Y. Fan and J. Ma, *Soft Matter*, 2011, 7, 9239-9247.
94. Y. Sun, Y.-L. Sun, L. Wang, J. Ma, Y.-W. Yang and H. Gao, *Microporous and Mesoporous Materials*, 2014, 185, 245-253.
95. Z. Yufang, S. Jianlin, S. Weihua, C. Hangrong, D. Xiaoping and R. Meilin, *Nanotechnology*, 2005, 16, 2633.
96. Z. Feng, Y. Li, D. Niu, L. Li, W. Zhao, H. Chen, L. Li, J. Gao, M. Ruan and J. Shi, *Chemical Communications*, 2008, DOI: 10.1039/B804594A, 2629-2631.
97. J.-G. Wang, F. Li, H.-J. Zhou, P.-C. Sun, D.-T. Ding and T.-H. Chen, *Chemistry of Materials*, 2009, 21, 612-620.
98. Y. Wang, Y. Yan, J. Cui, L. Hosta-Rigau, J. K. Heath, E. C. Nice and F. Caruso, *Adv Mater*, 2010, 22, 4293-4297.
99. A. P. Esser-Kahn, S. A. Odom, N. R. Sottos, S. R. White and J. S. Moore, *Macromolecules*, 2011, 44, 5539-5553.
100. A. P. R. Johnston, G. K. Such and F. Caruso, *Angewandte Chemie International Edition*, 2010, 49, 2664-2666.
101. K. Kempe, S. L. Ng, K. F. Noi, M. Müllner, S. T. Gunawan and F. Caruso, *ACS Macro Letters*, 2013, 2, 1069-1072.

Journal of Materials Chemistry B Accepted Manuscript

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102. K. Liang, G. K. Such, Z. Zhu, Y. Yan, H. Lomas and F. Caruso, *Advanced Materials*, 2011, 23, H273-H277.
103. A. N. Zelikin, J. F. Quinn and F. Caruso, *Biomacromolecules*, 2006, 7, 27-30.
104. K. Kempe, S. L. Ng, S. T. Gunawan, K. F. Noi and F. Caruso, *Advanced Functional Materials*, 2014, 24, 6187-6194.
105. X.-M. Xia, P. Yu, N. Peng, Y. Zhang, Y.-N. Xue, R.-X. Zhuo and S.-W. Huang, *Journal of Controlled Release*, 2011, 152, Supplement 1, e101-e103.
106. M. Okumura, M. Yamamoto, H. Sakuma, T. Kojima, T. Maruyama, M. Jamali, D. R. Cooper and K. Yasuda, *Biochim. Biophys. Acta, Mol. Cell Res.*, 2002, 1592, 107-116.
107. A. Rempel, S. P. Mathupala and P. L. Pedersen, *FEBS Lett.*, 1996, 385, 233-237.
108. T. Levy, C. Déjugnat and G. B. Sukhorukov, *Advanced Functional Materials*, 2008, 18, 1586-1594.
109. B. G. De Geest, A. M. Jonas, J. Demeester and S. C. De Smedt, *Langmuir*, 2006, 22, 5070-5074.
110. U. Manna and S. Patil, *ACS Appl Mater Interfaces*, 2010, 2, 1521-1527.
111. B. Koppolu, M. Rahimi, S. Nattama, A. Wadajkar and K. T. Nguyen, *Nanomedicine*, 2010, 6, 355-361.
112. I. N. ANTAL, J. DREDÁN, P. HAJNAL, I. Klebovich, M. B. LENGYEL and P. SZEGŐ, Google Patents, 2012.
113. W. Xu, P. A. Ledin, F. A. Plamper, C. V. Synatschke, A. H. E. Müller and V. V. Tsukruk, *Macromolecules*, 2014, 47, 7858-7868.
114. S. Mandal, N. Rosso, C. Tiribelli, G. Scoles and S. Krol, *Soft Matter*, 2011, 7, 9424.
115. Y. Zhu, W. Meng, H. Gao and N. Hanagata, *The Journal of Physical Chemistry C*, 2011, 115, 13630-13636.
116. O. Khanna, M. L. Moya, H. P. Greisler, E. C. Opara and E. M. Brey, *Am J Surg*, 2010, 200, 655-658.
117. D. P. Go, A. Hung, S. L. Gras and A. J. O'Connor, *J Phys Chem B*, 2012, 116, 1120-1133.
118. D. P. Go, S. L. Gras, D. Mitra, T. H. Nguyen, G. W. Stevens, J. J. Cooper-White and A. J. O'Connor, *Biomacromolecules*, 2011, 12, 1494-1503.
119. C. Rhodes, N. MALAVIA, R. Jennings, R. LAXMA and B. NORMAN, Google Patents, 2014.
120. C. Büssing, M. Marchesan and M. Hummel, Google Patents, 2013.
121. WO2013021409A1, 2013.
122. A. Voigt and L. KRÖHNE, Google Patents, 2011.
123. W. L. Lee and S. C. J. Loo, *Journal of Drug Targeting*, 2012, 20, 633-647.

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