REVIEW

Drug Releasing Polymer Thin Films: New Era of Surface-Mediated Drug Delivery

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ABSTRACT Polymer films and coatings are among the popular and most successful tools to modulate surface properties of biomaterials, specifically tissue responses and fouling behavior. Over the past decade, a novel opportunity has been widely investigated, namely utility of surface coatings in surface-mediated drug delivery. In these applications, deposited polymer films act as both a coating to modulate surface properties and a reservoir for active therapeutic cargo. The field has recently accelerated beyond the proof-of-concept reports toward delivering practical solutions and established technologies for biomedical applications. This review briefly summarizes the recent successes of polymer thin films, specifically those constructed by sequential polymer deposition technique, in surface-mediated drug delivery.

KEYWORDS: polymers · thin films · sequential deposition · drug delivery · cell adhesion · gene transfer

Advances in healthcare and surgery, specifically novel opportunities associated with artificial transplants and stents[1] and tissue engineering using scaffold materials,[2,3] have given rise to a new research direction, namely biomaterial−tissue interaction.[4−6] While the first efforts in the field aimed to create benign, nonreactive interfaces, subsequent research undertakings increasingly considered beneficial interaction between implants and tissues, specifically aiming to facilitate development of particular cells, elicit cell specific responses, and achieve sustained drug delivery mediated from the surface. The latter possibility is particularly important for success of cardiovascular stenting and prevention of restenosis whereby delivery of drugs from the stent surface creates a higher local concentration of the antiproliferative and/or anti-inflammatory drugs through their local release at the site of action.[1,7] Until recently, polymer coatings were largely considered as tools to improve biocompatibility of the underlying matrixes which exhibited controlled release of therapeutic molecules. Diverse polymer coating techniques (physical adsorption, chemical grafting, *etc.*) are indeed among the best performing candidates in the area. Very recently, a novel opportunity has emerged whereby a polymer coating is used to achieve both, a control over cellular adhesion and proliferation and a controlled release of active therapeutics, from small drugs to proteins and nucleic acids (Figure 1). A particular success story relates to a sequential deposition technique in which interacting polymers are adsorbed or chemically bound to the surface in an iterative, layer-by-layer (LbL) fashion (Figure 2). Over the past two decades, a plethora of structurally diverse synthetic and natural polymers were used to create multilayered films through electrostatic assembly, hydro-

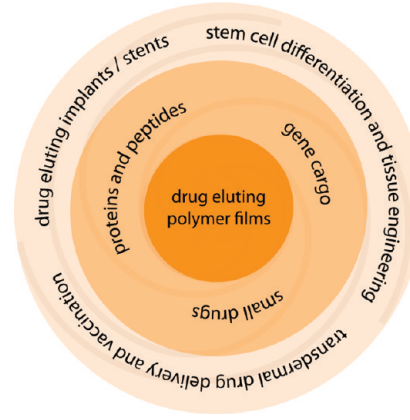

Figure 1. Polymer thin films were recently employed as reservoirs for diverse cargo, including small drugs, peptides, and intact full proteins and gene cargo, for their sustained surface-mediated release. These films have already proven useful in diverse biomedical applications including the creation of drug eluting stents, delivery of antibiotic and anti-inflammatory drugs, guided differentiation of stem cells, and transcutaneous delivery of vaccines and adjuvants.

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gen bonding, and covalent interactions producing coatings with thicknesses from several nanometers to tens of micrometers and even free-standing membranes with amazingly different surface and bulk properties, from highly stable to degradable coatings and materials responsive to diverse chemical and physical stimuli. The process is typically all aqueous, which is highly favorable for drug loading and cargo stability, and requires minimal if any surface preparation, the two characteristics making it highly attractive for biomedicine. Together with the instrumental simplicity of this technique, this has led to an avalanche-like development of LbL over the first decade of its existence, during which it documented the use of commercial and custom-made polymers of all colors and flavors. This blossoming field has been extensively reviewed from different perspectives[8−12] and its full characterization and presentation is beyond the scope of this review. Herein, we aim to describe the first successes of this technique in the surface-mediated drug delivery. We begin with modulation of cellular adhesion, then move to release and delivery of small cargo, delivery and presentation of proteins and oligopeptides, and finally surface-mediated gene transfer. We note that the field has undergone a significant growth, and we therefore focus on successful examples rather than proof-of-concept reports in the area, for an overwhelming overview of the latter we refer the readers to earlier reviews.

DISCUSSION

Cellular Adhesion and Proliferation. Modulation of cell adhesion to substrates and surfaces as well as materials fouling, that is, interaction with proteins and microorganisms, is of tremendous importance for diverse areas of biomedicine, from biosensing to drug delivery and tissue engineering. For the field of surface-mediated drug delivery, its relevance is 2-fold and it relates to the two distinctly different delivery strategies, namely (i) release of the cargo into surrounding media (e.g., bloodstream) for opportunistic uptake by cells and (ii) delivery directly to the cells cultured on the biomaterial surface, possibly in response to the substrate degradation by the cultured cells. In the former case, adhesion of cells to the drug releasing surface is not required and in fact can lead to a detrimental change in the drug release kinetics; it can therefore be desirable to use cytophobic, that is, adhesion resistant polymer films. In contrast, uptake of therapeutic cargo by the cultured cells requires that the latter undergo attachment to the substrate, and cell adhesive properties of a candidate coating become an asset. Among the many approaches to create polymer coating and modulate cell adhesion and proliferation, sequential deposition of polymers appears to stand out as a very simple and forgiving technique. Virtually any surface is amenable to polymer coating *via* LbL; a plethora of commercial and custom-made polymers is at one's disposal; if the surface does not attain the desired properties after one deposited layer one oftentimes only has to deposit additional layers until the desired effect is achieved; last but not least important is that surface characteristics can be reversed almost at will *via* deposition of additional layers. Together with the development of patterning techniques for thin films, we believe LbL has become a truly powerful tool for controlled adhesion and differentiation of cells. In a brief discussion below, we will limit ourselves to a few examples of polymer coatings constructed *via* the sequential deposition technique which are of importance for the subsequent discussion on surface-mediated drug delivery.

Over the past decade there has been a progressive increase in the understanding of cell−substrate interactions and the importance of their fine control for successful tissue engineering and other biomedical applications.[4] Recently, the importance of substrate mechanical characteristics has come into focus of research activities, and it was for example shown that mammalian cells preferred to attach to stiffer regions of the gels and even turned around in their migration patterns to crawl back to the stiffer parts of the gel.[13] Differentiation of naive mesenchymal stem cells was shown to be remarkably sensitive to the substrate elasticity,[14] and on substrates with elasticity resembling brain, muscle, and bone tissue these cells differentiated and expressed neurogenic, myogenic, and osteogenic markers, respectively. For applications like these, multilayered films offer a facile route to tweak their mechanical characteristics through the choice of constituting components and cross-linking steps and therefore are unique candidate materials to create surfaces with programmable cell attachment and differentiation. A very early contribution from Hubbell *et al.*[15] reported on the sequential deposition of poly-L-lysine (PLL) and alginate (ALG) onto proteinaceous surfaces which support adhesion and proliferation of hu-

VOCABULARY: sequential polymer deposition technique (layer-by-layer, LbL) – An approach to create surface adhered polymer film *via* alternate adsorption of interacting macromolecules onto support surfaces. This can be conducted on surfaces with varied curvature, from macroscopic and planar substrates to nanometer sized colloidal particles. Most typical examples describe the use of polymers which associate through electrostatic interactions and hydrogen bonding, although supramolecular complexes and nanoparticles are also popular candidates, as well as covalent linking of the constituents · **biodegradable polymers** – synthetic or natural macromolecules which undergo degradation of their backbone in response to environmental stimuli or as a result of an enzymatic reaction. Examples of the former include polymers with hydrolytic mechanism of degradation (*e.g.*, hydrolysis of ester, orthoester, acetal linkages in an aqueous environment) and reduction sensitive polymers (*e.g.*, disulfide-stabilized); enzymatically degradable polymers include polypeptides, polysaccharides, nucleic acids and other natural or nature derived macromolecules · **cell adhesion** – a process of cell attachment to a surface of a biomaterial or another cell which involves cellular proteins, receptors and ligands and in many instances is a requirement for cell proliferation, differentiation and migration, as well as intercellular recognition and higher organization of cells · **surface-mediated drug delivery** – delivery of therapeutic cargo from the surface of a biomaterial, typically an implant, to the surrounding cells, organs and tissues. Typical examples include (i) a controlled dissolution of sparingly soluble cargo from the surface; (ii) uptake of adsorbed or otherwise immobilized cargo by the cells cultured directly on top, and (iii) release and possibly facilitated uptake of therapeutic molecules by eroding surface coating.

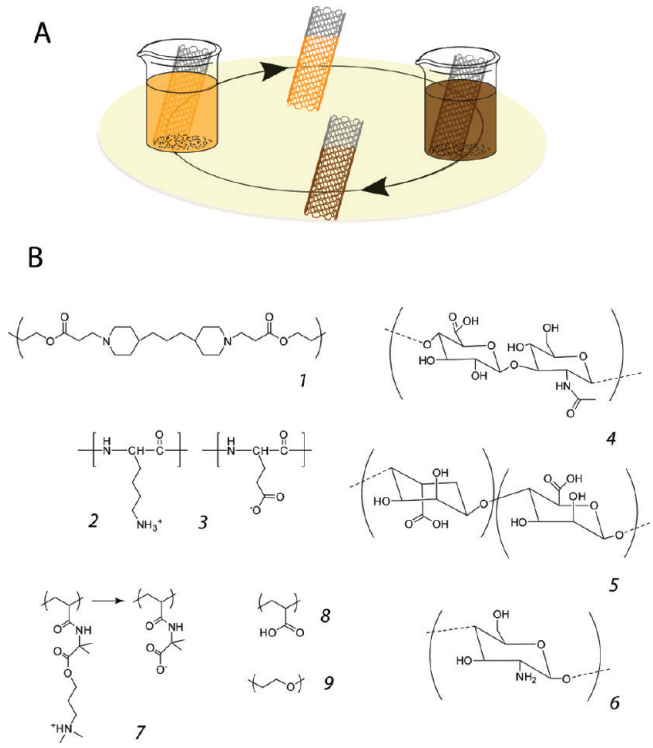

Figure 2. A schematic diagram of the sequential polymer deposition technique conducted on a surface of a model wire mesh (A) and representative synthetic polymers used in assembly of drug eluting thin polymer films (B). Sequential polymer deposition can be conducted through electrostatically facilitated assembly, hydrogen bonding, and other types of interactions on virtually any unprepared surface using substrate dipping (as shown), polymer spraying, spin coating, and other liquid application techniques, making it a facile approach for production of thin polymer films. For drug delivery, (bio)degradable polymer films are typically constructed using hydrolytically degradable polymers (*e.g.*, poly(β-aminoesters), representative example shown in structure 1), enzymatically degradable polymers (poly-L-lysine, 2; poly-L-glutamic acid, 3; hyaluronic acid, 4; alginic acid, 5; chitosan, 6), charge shifting polymers (7) as well as inherently unstable (cross-linked) hydrogen-bonded films, *e.g.*, made of poly(acrylic acid), 8, and polyethylene glycol, 9.

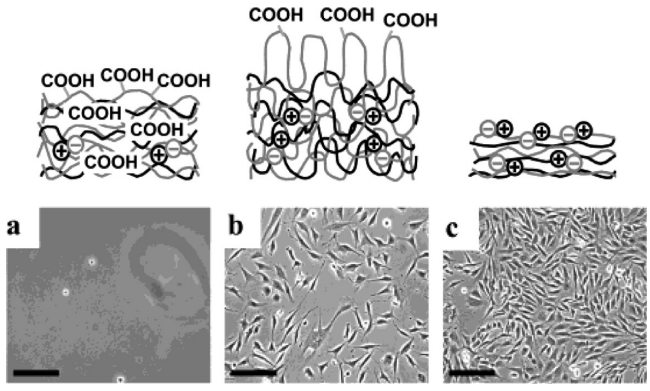

Figure 3. Mechanical compliance and softness of the surface are now recognized as powerful tools of control over its cell adhesive properties. These can be fine-tuned using polymer multilayered thin films *via* the choice of polymers, assembly conditions, and subsequent cross-linking of the films. In the example shown, the same polymers, PAA and PAH, were used to create polymer thin films with cytophobic properties (a), increasing cell adhesiveness (b) and those well suited for attachment and proliferation of mammalian cells (c) *via* the choice of assembly conditions rendering films gel-like (a) or highly stitched with ionic cross-links (c). Adapted from ref 17. Copyright 2003 American Chemical Society.

man fibroblasts. This polymer pair was chosen for its success in biomedical applications and specifically encapsulation of live cells.[16] Upon deposition of a certain number of bilayers, the surfaces became effectively cytophobic, and no cell spreading and proliferation was observed. This phenomenon was not linked to the cytotoxicity of surface coatings, and authors suggested that this effect is inherited with the hydrogel, soft nature of the coating. Another early example which remains among the most significant contributions to the field was delivered by Rubner *et al.* in 2003.[17] In this report, cell adhesion was controlled *not* through the identity of the polymers (poly(acrylic acid) (PAA) and poly(allylamine) (PAH) in all cases) but through the assembly conditions, that is, the architecture of the films (Figure 3). PAA−PAH thin films were overall similar to the tissue culture polystyrene (TCPS) with regard to adhesion of murine fibroblasts NR6WT with the exception of films highly enriched with PAA, that is, reminiscent of hydrogels. The latter were effectively cytophobic in nature regardless of the outmost layer, emphasizing the dominant influence of film architecture over surface chemistry on cell adhesion. As above, cytophobicity was not a result of cytotoxic effect, that is, the cells in fact never attached to the PAA-enriched multilayers. All tested combinations of polymers adsorbed proteins from bulk solution, including the cytophobic films, and were rather similar in their wettability characteristics. In other words, interaction with proteins and/or surface chemistry together could not explain the cytophobic nature of PAH−PAA multilayers enriched with PAA. Substituting poly(methacrylic acid) (PMA) for PAA yielded similar results, that is, PMA enriched multilayers were also cell resistant and were effectively cytophobic; this was also observed on PAH−poly(styrene sulfonate) (PSS) multilayers highly enriched with PAH. Authors conclude that cytophilic multilayers are densely stitched with ionic cross-links; cytophobic counterparts are loosely stitched, that is, possess few ionic cross-links. The latter exhibit increased swellability, which is a characteristic behavior of hydrogels that leads to their cytophobic behavior. In the follow up publication,[18] Rubner, van Vliet *et al.* documented a direct correlation between mechanical compliance of PAA−PAH multilayers and the observed cell adhesion. However, cell proliferation was found to be similar on all the substrates, and the final cell count was dictated by the initially adhered number of cells. It was also shown that mechanical properties have a greater effect than the chemistry and charge of the topmost layer. For example, PAH-terminated layers with markedly differed compliance were very different in supporting cell attachment.

Thus, mechanical properties of thin polymer films have proven to be a unique tool in modulating cell adhesion and proliferation. This early observation has in many ways directed further development of the field

and found proof in many subsequent publications, as described below. We believe that substrate softness and mechanical compliance are an important and powerful addition to the arsenal of tools of control over cell attachment and proliferation, which complements well the existing "classical tools", namely PEGylation of surfaces[19,20] and attachment of pro-adhesive peptides (*e.g.*, RGD[20,21]) and proteins (*e.g.*, fibronectin[22,23]), all of which are of course available in the context of sequentially deposited polymer coatings.

The spreading of vascular smooth muscle cells observed on collagen-coated PLL−hyaluronic acid (HA) multilayers was significantly more efficient on stiffer surfaces than their softer counterpart.[24] Chondrosarcoma cells (HCS2/8) adhered to the cross-linked, stiffer PLL−HA films while pristine noncross-linked films remained essentially cytophobic,[24] and for the PLL-terminated films the spreading cell area showed a strong correlation with the substrate Young's modulus.[25] Skeletal muscle cells C2C12 showed preferential adherence to the PLL−HA films with a higher cross-linking density, and there was a statistically higher number density of focal adhesion points observed on denser cross-linked films compared to their weaker cross-linked counterparts.[26] Furthermore, cell differentiation and formation of myotubes was only observed on well cross-linked substrates for which the expression of muscle specific proteins was found to be similar to that in myotubes formed on TCPS. This report thus clearly demonstrates the important role of polymer film rigidity in both cell adhesion *and* differentiation. Similarly, pristine PLL−HA films were ill suited as substrates for adhesion and proliferation of mesenchymal[27] and embryonic[28] stem cells (MSC and ESC, respectively) and in both cases required cross-linking to sustain cell adhesion. Adhered MSC were capable of differentiating into osteocytes and chondrocytes upon instruction *via* external chemical stimuli,[27] while for EMS, substrate stiffness influenced differentiation of cells into epiblast.[28] Cross-linking was also employed in an effort to increase adhesiveness of chitosan (CHI)−HA and CHI−ALG multilayers.[29]

Recently, a deeper analysis of substrate elasticity and its influence on cellular adhesion and proliferation was offered using PtK2 epithelial cells and PLL−HA films as substrates.[30] Polymer film rigidity was modulated *via* additional PSS−PAH layers deposited on the surface. As above, pristine PLL−HA films were found to be cytophobic and an increase in the substrate rigidity was accompanied by a progressive increase in the cell adhesion. Interestingly, substrate elasticity was shown to have a complex influence with a cascade of independent events being switched on or off upon a change in matrix properties. In particular, the contributions of this factor to regulation of transcription and replication were found to be uncoupled. For example, the cells that adhered to substrates with rigidity of ~50 kPa had arrested replication but not transcription. While it is probable that these findings (the exact elasticity parameters and their influence) are cell-type specific, they can have a high impact on drug delivery applications, specifically in delivery to the adherent cells through a substrate mediated modulation of their cellular activity.

From a different perspective, multilayer constituents were also explored as means to control cell attachment. Multilayered films constructed using (pseudo) natural biodegradable polypeptides, PLL and poly-L-glutamic acid (PGA), were among the early candidates used in these experiments.[31] While PLL terminated films were successful in supporting cell attachment and proliferation[31,32] their PGA counterparts showed no cell adherence.[32] These films later on found use in drug delivery applications, as will be discussed below. In what was among the first efforts aimed to *induce* rather than *suppress* cell adhesion, Ogier *et al.*[33] used multilayered films with different architecture and polymer terminating layer and monitored adhesion and proliferation of osteoblast human carcinoma cells (SaOS-2) and human periodontal ligament cells (PDL). Poly(ethyleneimine) (PEI), a cationic polymer with a well documented cytotoxicity, when deposited as a topmost layer did not allow the cell lines to reach confluency indicating toxic effects. In contrast, PSS, PAH, PGA and PLL all supported normal cell proliferation and morphology similar to that observed on cells cultured on TCPS. Effect of multilayers on cell attachment was evident within the first 20 min of incubation, and a greater number of cells were adhered to the polymer-coated surfaces than to TCPS. For positively charged polymers as topmost layers, analysis of cells cultured on multilayers showed a significant decrease in the production of an osteoblast phenotypic marker (ALP), being pronounced for PEI, noticeable for PAH and non-negligible for PLL (only for PDL cells, not SaOS-2). In contrast, for PGA and PSS terminated multilayers, this marker was expressed at a normal level. From the stand point of production of pro-inflammatory cytokines, results varied between the two cell types yet generally PSS was neutral (elicited no cytokine production) and PGA and PLL were also found to be promising coatings.

In their comprehensive study of multilayered polymer films for hepatocellular applications, Van Tassel *et al.*[34] assembled thin films using polypeptides (PLL;PGA); polysaccharides (CHI; ALG) and synthetic polymers (PSS; PAH) and used three different cells lines to monitor their adhesion and proliferation. A remarkable finding of this work is that HepG2 only reached confluence on PLL-terminated PLL−ALG films and PSS terminated PAH−PSS films while no other chemistry and polymer combination was suitable for this. It is surprising that polysaccharide films which are reminiscent of extracellular matrix and therefore expected to support cell attachment exhibited poor ability to support HepG2 adhesion, including the films constructed with galacto-

sylated sugars which are known to enhance hepatocyte attachment. Film topmost layer was shown to be important in cell attachment and differentiation, as evidenced by the differences between PSS and PAH terminated PSS−PAH multilayers. However, film surface charge did not appear to be a decisive factor, and of the two identified lead substrates for HepG2 adhesion and proliferation, one was terminated with a positive component (PLL) and one with a negative (PSS). Film rigidity was found important and an increased film cross-linking in PLL−ALG films was followed by a significant increase in HepG2 attachment and growth. However, no other film exhibited an improvement in performance upon an increase in rigidity thus suggesting that this is one of many contributing factors. It is important to note that this publication is in seeming contradiction with other reports in the field (*cf.* influence of the topmost layer and film rigidity; PLL−PGA multilayers for cell attachment, *etc.*). We do believe that this is a strong indication of cell specific effects and also possibly a result of differed assembly conditions. It is therefore fair to say that polyelectrolyte multilayers can both support the attachment and differentiation of cells and be altered nonadherent *via* assembly conditions and candidate materials.

In the discussion above, we aimed to demonstrate that polymer thin films are a powerful tool to control cell attachment and proliferation, specifically coupled with the ability of sequential deposition technique to be applied to virtually any surface without restriction to its geometry and origin. As an example of their practical utility, multilayered polymer films were used as coatings of blood vessels to improve their mechanical properties and accelerate endothelialization[35,36] or control of restenosis *via* minimized cell adhesion.[37] We expect that the success of these applications combined with the advances in drug releasing films described below can lead to novel opportunities in biomedicine and particularly tissue engineering. Yet another important technological possibility of this technique relates to the demonstrated opportunities for patterning and spatial control over cell adhesion both in two[38] and three[22] dimensional space and we believe it will likely have immediate ramifications for surface-mediated drug delivery. In an elegant example in 2D,[23] coculturing of multiple cell types was achieved using patterning based on cell adhesive/resistant polymer surfaces and an ease of the reversal of this property using additional polymer layers. Thus, HA islets were used as a cytophobic pattern with the first cell type seeded around HA-coated surfaces. Subsequent adsorption of collagen onto HA gives rise to a new cell adhesive surface and allows attachment of a second cell type and their coculturing, an important step toward engineering of multicell type tissues and organs. Patterning was also achieved using synthetic polymers (PSS and poly(dimethyldiallylammonium chloride) (PDADMAC)), primary cells, and exploiting nonproliferative characteristic of PDADMAC coated surface to primary neurons.[39] Patterning the polycation in this case was achieved *via* microcontact printing; initial seeding of neurons resulted in their adhesion to the PSS terminated areas only, subsequently seeded astrocytres adhered between the neurons thus giving rise to a final distribution of primary cells on the surface. In 3D space, the inherent layered structure of the LbL derived films allows the creation of multistrata films[40,41] and potentially confines differed cell types within their own niche. We believe that patterning techniques will complement well the developments in surface-mediated drug delivery using polymer thin films and enhanced the opportunities offered by this therapeutic approach.

Delivery of Small Cargo. Surface-mediated delivery of small molecule drugs is extensively studied within biomedicine. In the particular case of drug eluting stents it allows minimizing the undesired adhesion of smooth muscle cells to the surface of the implant and diminishing inflammatory responses through localized delivery of anti-proliferative and anti-inflammatory drugs, respectively.[1,7] As a strategy of controlled release of small cargo, polymer thin films are a young paradigm making first steps toward successful applications. Typically, immobilization and retention of (model) drug molecules are achieved *via* absorption into preformed multilayers, and this strategy was indeed adopted for drug delivery. For example, was absorbed into the preformed PLL−HA multilayered films[42] from its aqueous pH 6.5 solutions and was shown to remain adsorbed without passive release in the presence of 0.15 M NaCl for at least 4 days. The drug was available to the adhering cells and the viability of the cultured cells was significantly reduced. Similarly, antibacterial chlorhexidine digluconate was absorbed into PLL−PGA multilayers, and the films inhibited bacterial growth.[43] In another approach, gentomycin (GS) was used as a constituting component of a polymer-based thin film constructed using biodegradable poly(β-aminoesters) and HA.[44] The success of film buildup and incorporation of GS required that layers of GS−HA are deposited between the layers of the polycation and HA, in which case stable coatings and even free-standing films (over a hundred deposited polymer layers) were formed. The films released GS with kinetics controlled by the structure of a chosen poly(β-aminoester), and both the coatings and free-standing films were inhibiting bacterial growth while remaining nontoxic to mammalian cells. While these and other[45] reports demonstrate that some low molecular weight drugs can be loaded into polymer thin films for their surface-mediated delivery, we believe that success of these undertakings is limited to specific examples. Furthermore, it may be increasingly hard to engineer specific release profiles in such systems, especially if codelivery of multiple drugs is considered.

A more advanced possibility as offered by the field of sequential polymer deposition employs host or carrier compartments which are incorporated into the coating and retain small cargo for controlled release. Further decoration by subsequent polymer layers can achieve specific interaction with surrounding tissue and cells, as described in the previous section, and give rise to multifunctional polymer coatings. In an early example, PAH and aldehyde-functionalized poly(ethylene oxide) (PEO)−poly(lactic-co-glycolic acid) (PLGA) micelles[46] were used to construct a multilayered architecture *via* repetitive chemical reaction between amines and aldehydes. Micelles acted as hosts to hydrophobic cargo and cumulative loading was proportional to the number of layers deposited on the surface. Incorporation of copolymer micelles into thin films was subsequently achieved based on noncovalent interactions, specifically electrostatic[47] and hydrogen bonding,[48] including scenarios where both negatively and positively charged components are micellar.[49,50] Hydrophobically modified polysaccharides, which are known to form supramolecular structures and solubilize hydrophobic cargo, were also incorporated in the polymer thin films.[51] Incorporation of intact liposomes into the polymer films has been achieved,[52,53] yet it commonly presents itself with a challenge and special techniques are employed for stable anchoring of liposomes without their rupturing. One of these approaches is based on prefunctionalization of liposomes with polyelectrolytes which contribute to stiffening or increased rigidity of liposomes and also provide higher affinity to the counter-charged polymers.[54] Another strategy relies on the use of custom synthesized cholesterol-modified polymers and their anchoring with liposomes *via* insertion of cholesterol into the lipid membrane.[55,56] The latter process is fast, proceeds regardless of liposome composition, and is largely insensitive to the presence of other solutes in the media thus making a facile and flexible strategy of liposome decoration with polymers. This approach allowed subsequent interaction of functionalized liposomes with polymers *via* both electrostatic and hydrogen bonding and provided stable incorporation of these potential drug carriers within polymer thin films. Thus, a range of successful supramolecular carriers of small drugs have been incorporated into the polymer thin films. While their use in drug delivery remains largely unexplored, a few first successful examples have appeared recently, and these are discussed here in detail.

Hydrophobic bactericide triclosan was incorporated into polymer thin films formed by PAA as a negative component and positively charged linear−dendritic block copolymer micelles with poly(propylene oxide) (PPO) hydrophobic core and poly(amidoamine) (PAMAM) dendrimer as corona.[57] This architecture exhibited sustained drug release over a period of at least 2 weeks albeit without an external trigger or stimulus, that is, in a diffusion controlled manner. Another approach to delivery of small cargo using block copolymer micelles, in this case positively charged poly(2-vinyl ethylpyridinium)−poly(ϵ-caprolactone) (P2VEP−PCL) micelles, made use of heparin or dextran sulfate as anionic components of the film and poly(β-aminoesters) used in alternation with micelles providing for degradation of the multilayered architecture.[58] As polysaccharides are also bioactive compounds, this system represents an example of codelivery of multiple structurally different candidate drugs.

In contrast to electrostatically stabilized multilayered polymer thin films, hydrogen-bonded counterparts with a polymeric acid (PAA, PMA) acting as a hydrogen donor are only stable in acidic media and spontaneously deconstruct at pH levels above pK_a of the polyacid. In other words, these films are inherently unstable in the typical working range of the pH employed in biomedicine, pH 6−8. While this feature certainly limits the utility of pristine H-bonded films, in many instances it was turned into a benefit. Specifically for drug release applications, a promising approach relies on the cross-linking of multilayered films using linkages with well-characterized degradation behavior. For surface-mediated delivery of triclosan, PAA multilayers were assembled using PEO−PCL micelles as reservoirs for the hydrophobic drug and also H-bonding acceptor.[59] To render thin films stable, these were thermally cross-linked into anhydride-stabilized polymer films with degradation and drug release kinetics over a period of several days, and released triclosan was effectively inhibiting bacterial growth.

Cyclodextrins (CD) are among the most well studied hosts for diverse cargo, and these were first incorporated into multilayered films for delivery of piroxicam, a nonsteroidal anti-inflammatory drug with a limited solubility in water.[60] Piroxicam inclusion complexes with a negatively charged CD were incorporated into PLL−PGA multilayered thin films and exhibited a potent anti-inflammatory action (inhibition of pro-inflammatory cytokine production by THP-1 cells) over a period of at least 12 h. Positively charged CD derivatives were also shown to retain their "molecular chaperone" effect, that is, ability to facilitate delivery of therapeutic molecules, on an example of PLL−PGA multilayers and delivery of lipopolysaccharides (LPS).[61] In absence of CD, LPS from within the polymer films was unable to elicit production of pro-inflammatory cytokines by THP-1. In contrast, when LPS was incorporated into these films with CD molecules, the coating exhibited marked bioactivity. These multilayers in a combination with CD have also proven useful in the delivery of lipid A antagonist and achieved inhibition of LPS-induced production of pro-inflammatory cytokines.[62] PLL−CD conjugates were later explored as carriers for risedronate, a bisphosphonate drug used in the treat-

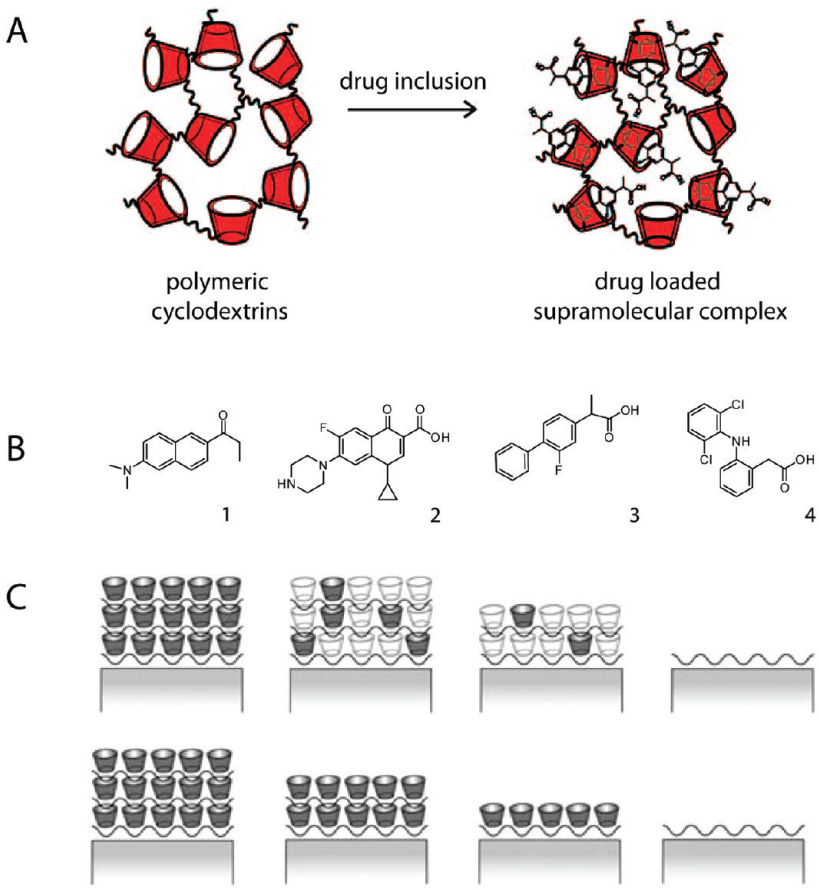

Figure 4. Successful incorporation of small molecule drugs and their sustained release can be achieved using polymer thin films and using an anionic supramolecular complex of cyclodexrins (A) which act as hosts for drug molecules (B: Fluorescent chemical probe prodan, 1, antibiotic drug ciprofloxacin, 2, non-steroidal anti-inflammatory drugs flurbiprofen, 3, and diclofenac, 4), and a biodegradable polycation to ensure hydrolytic degradation of the films. (C) Of the two proposed mechanisms of drug release, diffusion controlled (top) and surface erosion (bottom), the latter was shown to be dominant which led to a near linear drug release pattern with film degradation kinetics being dependent on the polycationic constituent of the film. Adapted with permission from ref 64. Copyright 2009 Wiley-VCH Verlag GmbH & Co. KGaA.

ment of bone metastasis for delivery, from within the multilayered films with PGA.[63]

A recent report from Hammond group[64] appears to deliver the most advanced opportunities and the greatest level of control over surface-mediated delivery of small therapeutic cargo using polymer thin films. A negatively charged polymeric cyclodextrin host was used in a sequential deposition with hydrolytically degradable poly(β-aminoesters), Figure 4. Several antibiotic and anti-inflammatory compounds were used as guest molecules and were delivered from these polymer thin films with controlled, burst-free, near linear release kinetics over at least 2 weeks. The two important aspects of this delivery paradigm are that the films undergo a surface erosion degradation thus allowing a linear release profile, and the choice of a poly(β-aminoesters) allows tweaking the release kinetics to suit the needs of particular applications. Furthermore, reported release kinetics was not dependent on the cargo small molecule but was defined by the polymer constituents of the thin film, thus opening an opportunity to closely control the release of two or more drug candidates from the same thin film for a combination therapy and/or multiagent treatment.

Delivery and Presentation of Protein and Peptide Cargo. Controlled release and presentation of protein and/or peptide cargo has become a pivotal technique in tissue engineering and stem cell research, as well as other biomedical applications. Proteins are among the main structural components of living organisms, and their functions span the widest possible spectrum encompassing inhibition and stimulation of cellular adhesion, pro- and anti-inflammatory action, pro- and anti-differentiation and cytotoxic effect, *etc*. In essence, protein molecules serve as building blocks, as signaling molecules, and as chemical stimuli and have many other functions in every organism. It is therefore of utmost importance and therapeutic potential to gain control over their presentation and delivery, specifically mediated on the surface of implanted materials. In the simplest form, proteins and peptides, possibly in a form of their conjugates, can be adsorbed onto surfaces, as used in tissue engineering and some other applications.[65] These applications are often successful in their own right, yet multilayered architecture of thin films allows an enhanced deliverable payload and an intriguing possibility of time-delayed presentation of multiple stimuli. Some of these prospects are already fulfilled and some are awaiting their implementation, and the following examples aim to demonstrate the current state of art.

The year 2001 appears to be the birth mark for delivery and presentation of protein and peptide cargo using polymer multilayers.[31] A peptide hormone, α-melanocortin (α-MSH) was coupled to a carrier PLL and used to construct multilayered films with PGA whereby PLL−α-MSH was incorporated at different depths. Production of cyclic adenosine monophosphate (cAMP) was used as an initial test of the peptide functionality within the multilayers and was measured after 2 h of incubation of B16-F1 cells on the thin films. Polymer films with PLL−α-MSH on the surface and also embedded underneath as many as 10 subsequent layers of PLL−PGA were active and elicited cAMP production comparable to that achieved by the peptide in solution. Cellular response to the α-MSH on a longer scale (after 4 days of cell culturing on top of the multilayers) was evaluated monitoring induced production of melanin which was strikingly similar for the PLL−peptide presented on the surface of the film as well as embedded

under 25 layers of PLL−PGA. This report also postulates that multilayers retained the biological activity of their cargo over at least a month when air-dried, thus contributing to the practical attractiveness of this technique. PLL−PGA multilayers containing PGA−α-MSH bioconjugate were further evaluated *in vivo* as coatings of tracheal prosthesis and showed promise in eliciting a production of anti-inflammatory cytokine, interleukin-10.[66]

Heparin has been used as an acceptor and carrier for growth factors in diverse tissue engineering applications. It is a nature inspired strategy of protein immobilization and localization, and it was among the first successful tools in the hands of biomaterial scientists. It was also used in the context of polymer thin films, specifically as a negatively charged coadsorbed component of an LbL film with acidic fibroblast growth factor (aFGF).[67] The main effect of delivered aFGF is an enhanced rate of proliferation of fibroblasts, and this was indeed the observed result. Another growth factor, VEGF, was successfully adsorbed onto polymer thin films for presentation to the cultured human umbilical vein endothelial cells (HUVEC) which resulted in an enhanced cell proliferation.[68] However, both pristine thin films and the VEGF-functionalized counterpart exhibited a global activation of HUVEC cells to a similar level (monitored through the total cell content of tyrosine phosphorylated proteins), thus implying that further studies are required to verify utility of the proposed architecture for growth factor delivery and presentation. Brain-derived neurotrophic factor (BDNF) and chemorepulsive Semaphorin 3A (Sema 3A) proteins were shown to retain their biological activity when adsorbed onto polymer thin films leading to an increased and a decreased proliferation of neurons cultured on these films, respectively.[69] Basic fibroblast growth factor adsorbed onto polymer thin films also retained its activity, and its use led to an increased number of attached and proliferating photoreceptor cells.[70]

PLL−PGA films, that is, coatings constructed using biodegradable polypeptides, were shown to be a suitable platform for presentation and delivery of active peptides and protein molecules to the cultured cells in a number of reports. Protein A retained its activity embedded within PLL−PGA multilayers and induced production of cytokines by monocytic THP-1 cells cultured on the surface of the film.[71] Remarkably, even when initially embedded under 20 bilayers of PLL−PGA, protein A was internalized by cultured cells. These multilayers were also used as reservoir for an antifungal peptide[72] which remained active within the films: the peptide-functionalized polymer coatings inhibited the growth of yeast (*Candida albicans*) and stopped proliferation of a filamentous fungus (Neurospora crassa) while remaining nontoxic to mammalian cells.

PLL−PGA films have proven useful for delivery of bone morphogenic protein BMP4, pro-apoptosis factor, and its inhibitor, Noggin, to a cultured first lower molar tooth.[73] Visual observations suggested that both proteins remain active and can induce and inhibit apoptosis, respectively, when released from the polyelectrolyte multilayered films. Subsequent attempts to deliver the two factors stepwise, that is, induce and then inhibit apoptosis or vice versa, were encouraging but need further verification. The same multilayered films containing cyclodextrins were used for delivery/presentation of two growth factors, BMP-2 and TGF-β, performed to control differentiation of embryonic bodies into cartilage and bone tissue.[74] The authors suggest a synergistic effect was achieved *via* incorporation of two growth factors, and little if any effect is noticed when only one protein is used. The suggested mechanism of their action is that *via* a contact between the cells and functionalized thin films and not *via* a proteins release into bulk solution. Other studies in the field have also confirmed that BMP-2 can be incorporated into multilayers at different locations or depth of protein embedding and the protein remains biologically active.[75]

In another study, cross-linked PLL−HA thin films were used as a biomimetic reservoirs to immobilize rhBMP-2 *via* adsorption into a preformed polymer film.[76] Limited solubility of rhBMP-2 at pH 7 allowed infiltrating the protein at pH 3 and then effectively entrapping it within the films upon the change in pH to 7. This strategy also minimized the characteristic burst release of the cargo. C2C12 myoblasts were then used to ascertain the activity of entrapped rhBMP-2: while on cross-linked growth-factor-free polymer films, as expected, the cells successfully formed myotubes, protein loaded films sustained differentiation of cells into a osteogenic lineage (Figure 5). Remarkably, the films maintained activity over at least 12 days and 3 successful replating of cells. The proposed mechanism of action for rhBMP-2 delivered *via* this technology is not a release of protein into bulk solution but one involving a contact of cells with the protein within the matrix and possibly degradation of the multilayers by cell enzymes. We believe that this report significantly increases the attractiveness of polymer thin films for tissue engineering. In a follow-up publication,[77] these authors showed that incorporating heparin as a component of thin films does not provide for enhanced rhBMP-2 retention, and growth factor activity is insensitive to the film composition. PLL−HA films were also superior in the durability of the BMP-2 action and supported a prolonged protein activity.

A strategy to facilitate incorporation of proteins into the multilayered films *and* assist their cell entry upon release from the multilayers involves conjugation of proteins to oligoarginine, a well-known transduction oligopeptide.[78] To this end, RNase A was conjugated with a 9-amino-acid arginine sequence, which

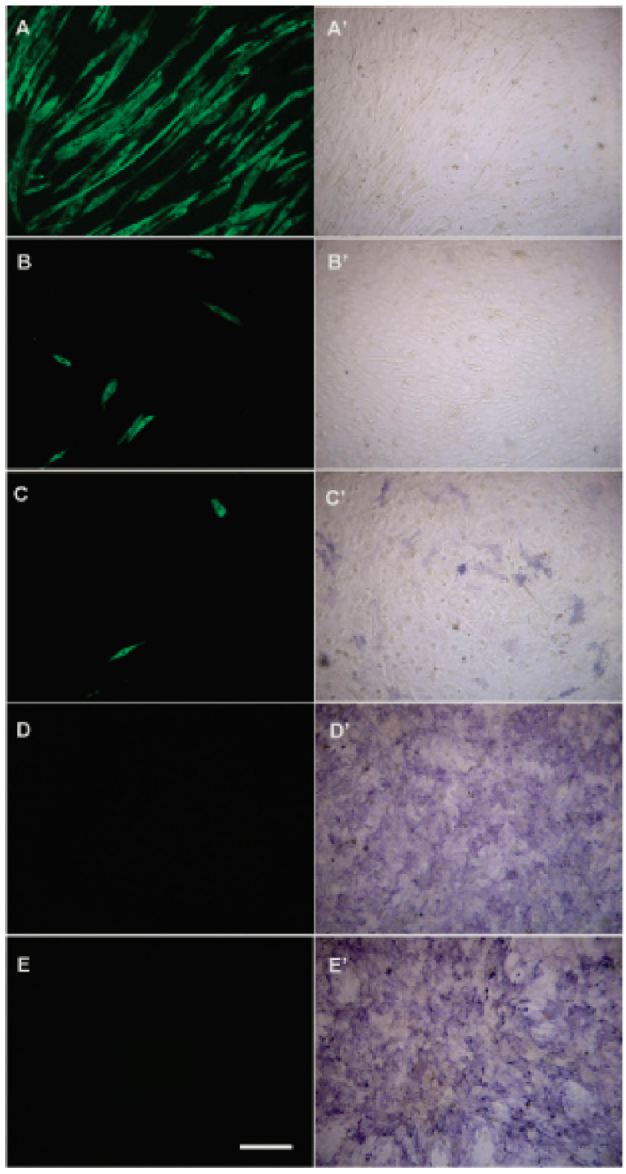

Figure 5. Recombinant human bone morphogenic protein 2 (rhBMP-2) was successfully delivered from a cross-linked PLL−HA multilayered polymer thin film to the cultured myoblasts in a dose-dependent manner (increasing concentration from A to E) resulting in an increase in cell production of alkaline phosphotase (right column, histochemical staining) and a decrease of troponin T (left column, immunochemical staining), both observations suggesting that myoblasts undergo differentiation into osteoblasts. Reprinted with permission from ref 76. Copyright 2010 Wiley-VCH Verlag GmbH & Co. KGaA.

endowed the conjugate with sufficient charge to perform sequential deposition with PSS. Buildup of the film was regular and efficient over at least eight bilayers, which allowed controlling the amount of incorporated protein. Regretfully, upon exposure to the physiologically relevant conditions (phosphate buffered saline (PBS)), the multilayers disintegrated and rapidly released most of their payload into bulk solution thus not permitting controlled release of the proteins from these constructs. However, the released protein was active and was successfully internalized by cells cultured in the presence of protein-releasing constructs.

Degradable poly(β-aminoesters) were also recently employed for surface-mediated release of a model protein cargo, specifically lysozyme.[79] At assembly conditions, lysozyme and the degradable polymer of choice are of similar charge, and for successful assembly a negatively charged biopolymer, heparin sulfate or chondroitin sulfate, was used. After a lag phase, the film buildup was linear, which allows facile control over protein loading and thus dosage of the therapeutic. Time tuned and sustained release of the protein was achieved over at least 10−30 days depending on the chosen poly(β-aminoester), the total amount of released cargo being a function of number of deposited polymer layers.

Surface Mediated Delivery of Nucleic Acids. Delivery of DNA from the surface has been introduced as a strategy to create a high local concentration of nucleic acid and achieve its localized delivery to the surrounding, possibly adhered cells.[80−82] The strategy has proven to be successful not only *in vitro* but also *in vivo* for DNA delivery from the surface of implantable stents. Levy *et al.*[83] incorporated DNA into a poly(lactic-*co*-glycolic acid) drug eluting coronary stent coating. Nucleic acid was released from the stent over a period of at least 10 days in a functional form and gave significant transfection levels *in vivo*. In a different approach, plasmids were immobilized within polymer gel drops, dried, and subsequently exposed to a lipid transfection agent, all of which was performed in a well-free array format. Subsequent seeding of cells and their proliferation on top of the spotted array afforded cells locally transfected with different nucleic acids, an approach with a great promise in high throughput screening and analyses.[84] Adenoviral vectors were immobilized onto surfaces of cardiovascular stents to give rise to a platform with efficient gene transfer and therapeutic benefit.[85] Complexation with a polycation is among the most well established tools of gene transfer and was adopted for surface-mediated delivery by several groups. To this end, polycation−DNA complex particles are adsorbed[86] or otherwise immobilized[87] onto a surface with subsequent cell seeding onto this surface. Upon contact, adherent cells internalize DNA which results in a pronounced level of transfection, often far exceeding that achieved by the same tool of gene transfer in solution.[88] The above examples provided a high level of motivation to adapt the sequential deposition technique and realize the potential of this approach in surface-mediated delivery of nucleic acids.

From a polymer chemistry and physics standpoint, DNA is a highly charged polyanion with typical attributes of a negatively charged macromolecule including association with positively charged counterparts, such as polymers, particles, and macroscopic surfaces. The development of the sequential deposition technique was accompanied by an understandable rise in the interest to incorporate DNA into the multilayers,

and there is currently a hefty collection of examples of generic nucleic acid used as a component of these thin films. Of specific interest for drug delivery are the examples where multilayers are suited to release the active nucleic acid, and this has been achieved *via* the use of hydrolytically degraded polymers and also enzymatically degradable natural polymers. The first of these approaches was pioneered and extensively explored by David Lynn *et al*. who also provided several first-handed reviews of the subject.[89,90] Starting with a 2004 report,[91] this team investigated the use of hydrolytically degradable poly(β-aminoesters) in the buildup of multilayered thin films with functional DNA. As with other polymers, the number of deposition steps and solution conditions (pH, presence of low molecular weight electrolyte) were parameters of effective control over the overall polymer film thickness and the amount of incorporated nucleic acid. Incubation of the multilayered films in PBS over 20−25 h resulted in a gradual release of DNA from the multilayers into bulk solution as a result of hydrolytic degradation of a polycation. Nucleic acid retained its function, as evidenced by transfection studies using collected DNA and commercial transfecting agents. This team has subsequently gained control over release kinetics, investigated the mechanism of DNA release,[90] and successfully performed multilayer assembly on the intravascular stents,[92] all of which contributes to the overall characterization of the system. The authors demonstrated successful uptake of released DNA by the cells cultured in the presence of eroding multilayers (under the quartz slide with deposited thin film)[93] which is important in the context of drug delivery from the surface of implantable stents. Furthermore, time delayed uptake of two different plasmids released from the same multilayer film was demonstrated highlighting the possibility to codeliver multiple stimuli with a fine control over delivery time (Figure 6).[94] A recent development from this group relates to the use of "charge shifting" polymers,[95] that is, polycations with side chains loosing or reversing their charge with time. The use of these polymers allowed significantly extending DNA release time (up to 90 days), and it was also possible to release multiple plasmid DNA in a time delayed fashion.

Other approaches to DNA release from multilayers include the use of polypeptides, specifically PLL, which allows releasing nucleic acid into the surrounding milieu upon digestion of PLL by chymotrypsin.[96] Attainable degradation times reported are on the order of 10−40 h,[96] and could be controlled using amine cross-linking reagents, such as glutaraldehyde.[97] The use of reductively sensitive polypeptides as partners to DNA in the multilayered thin films was reported as a strategy to release DNA in response to disulfide cleaving reagents, specifically dithiothreitol.[98] Oligonucleotide-containing polyelectrolyte complex particles were released from degrading polymer thin films based on PAA and PEO which are inherently unstable at pH 7. siRNA-PEI particles were stamped and patterned on the surface of these films for localized and patterned gene delivery to the cells cultured underneath the drug releasing surface.[99] In this example the polymer film as such does not facilitate gene delivery but aids in the localization of deliverable payload. siRNA was also released from the multilayered thin films with phosphorylcholine polymer.[100]

The history of nucleic acids being incorporated into the multilayered polymer films and successfully used in surface-mediated transfection is very recent and starts with a 2005 report by Yamauchi, Kato, and Iwata.[101] Plasmid DNA was used as an integral component of a multilayered thin film with PEI used as a positively charged counterpart. Within these films, DNA is stably immobilized and no release of nucleic acid was registered from the surfaces, unless an electric pulse was delivered to the underlying electrode. This electroporation technique resulted in an effective transfection of cells cultured on the polymer films and expression of the delivered DNA. Also in 2005, Meyer, Ogier, *et al*.[102] used DNA−PEI polyplexes and adsorbed these onto the surface or embedded them underneath HA−CHI multilayers. These constructs were effective in delivering a nucleic acid payload to the Huh-7 cells cultured on top. To the best of our knowledge, this was the first example of cells cultured on the multilayered thin films made of biodegradable polymers and used for successful surface-mediated gene transfer.

In 2006, the potential of thin films to deliver therapeutics in a time-controlled and time-delayed fashion was demonstrated, specifically for the case of gene delivery.[103] PLL−PGA films were used as an underlying surface onto which cationic cyclodextrin and DNA were sequentially deposited. Using visual observation only, it is suggested that this mode of DNA delivery yields a 100% transfection efficiency, that is, all the cells cultured on top of the DNA containing multilayers contained reporter gene. It is further suggested that enzymatic degradation of thin films is responsible for the release of functional DNA. Interestingly, DNA incorporated into PLL−PGA films in the absence of cationic cyclodextrins is reported to have no transfection efficiency. This highlights the importance of cyclodextrins in the reported architecture but also suggests a narrower utility of these multilayered thin films for gene delivery. Nevertheless, the reported system was effective and demonstrates a further benefit of the sequential deposition technique, namely the possibility to incorporate cargo at varied depths within the multilayered films and thereby control the release of cargo in time. To this end, two plasmids were incorporated between layers of cyclodexrin within the PLL−PGA films. Both plasmids were successfully delivered to the cultured cells and expression of plasmids in time correlated well

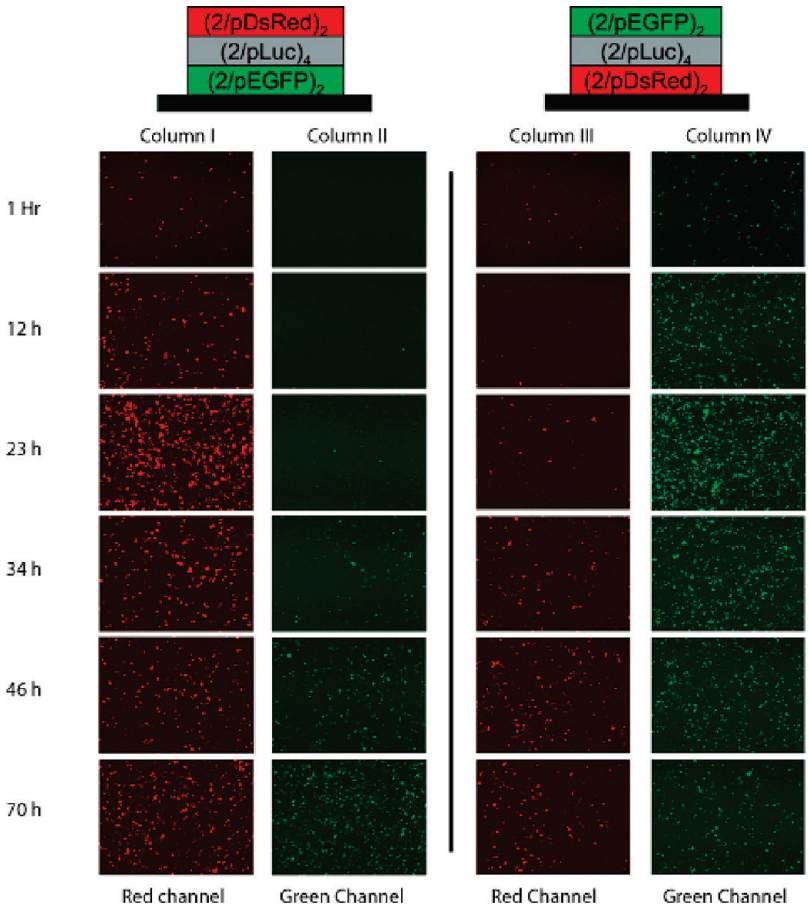

Figure 6. Representative fluorescence images of transfected COS-7 cells demonstrating time delayed release of two plasmid DNA from the multilayered polymer thin films. Plasmid DNA encoding for enhanced green fluorescent protein (pEGFP, green channel) and red fluorescent protein (pDsRed, red) were incorporated into multilayered films built using biodegradable poly(β-aminoesters) and positioned at a different relative position (pEGFP below pDsRed, columns I and II; pDsRed below pEGFP, columns III and IV); hydrolytic degradation of multilayers resulted in a release of DNA which was collected and used to transfect COS-7 cells using a commercial transfecting agent. Observed fluorescence of cells speaks toward the amount of DNA released from the thin film and therefore substantiates the time delayed release of two plasmids. Reprinted from ref 94. Copyright 2007 American Chemical Society.

with the difference in their positioning within the film architecture. In a follow up publication,[104] this team further developed DNA delivery mediated by the multilayered films and also ascertained the intracellular trafficking pathways of the internalized gene cargo. PLL and HA were chosen to construct the biodegradable polymer multilayers; PLL, cyclodextrin, and their conjugate were the tested gene carriers, and DNA was incorporated into the multilayers as a polyelectrolyte complex particle (not free DNA adsorbed within the multilayer). In accord with their previous report, surface-mediated transfection yielded higher efficiencies when compared to the same carrier used for solution-based gene transfer. HeLa cells cultured directly on top of the polymer films effectively internalized DNA from the films, as evidenced by the expression of a reporter gene. It is concluded that cell entry of PLL−CD complexes with DNA (as well as PLL−DNA and CD−DNA complexes) released from the multilayers) proceeds *via* a nonendocytic pathway, which is thought to be the underlying reason for an observed high level of transfection.

In another report, DNA was successfully delivered to the cells cultured on top of the films assembled with free nucleic acid and galactosylated chitosan as a polycation.[105] An important finding of this study is that DNA is released from the surface in a form of polyelectrolyte complex particles with chitosan, which can potentially benefit subsequent DNA cell entry. In HEK293 cells, similar levels of transfection were observed using these films and the ones built using pristine chitosan. In contrast, significantly higher levels of transfection were observed in HepG2 cells which express galactose receptor, and this led to a conclusion that cell recognition is also possibly an important factor in the surface-mediated transfection.

Dimitrova, Ogier, *et al.*[106] reported a strategy of surface-mediated gene transfer using bioactive adenoviral vector (Ad) delivered using multilayered polymer films. In an extensive study, delivery using several chemistries of the films and varied film architectures was investigated to reveal that this platform can be useful for delivery of adenoviruses. An important finding of this work was that Ad entry was observed in cells with and without specific receptor for this Ad, which was in stark contrast with a solution-based transduction. This team also delivered a 2008 pioneering effort of using polyelectrolyte multilayers for delivery of siRNA and success in RNAi.[107] A cushion of PLL−PGA film was used to adsorb siRNA complexes with PEI followed by separating layers of HA−CHI−HA. Multiple iterations of adsorption of siRNA−PEI followed by HA−CHI−HA allow an increased amount of incorporated nucleic acid, a benefit delivered by the sequential deposition technique. Delivery of antiviral siRNA to the cultured Huh7.5 cells containing replicating hepatitis C virus was sustained for a period of at least 12 days making it superior as compared to the solution-based delivery methods (liposomes, electroporation, PEI in solution) tested side by side with the multilayers. Furthermore, siRNA delivery from thin films was shown to provide inhibition of cell *infection* with the introduced virus. Therefore this technique is shown useful not only for the treatment of HCV but also in the inhibition of infection. It is further demonstrated that the mechanism of siRNA release is that related to the cellular enzymes degrading the multilayered films. We believe this report delivers a whole new avenue of use of polymer thin

films in biomedicine, specifically in RNAi and antiviral treatment.

The year 2009 saw several highly successful and promising reports of surface-mediated gene transfer using multilayered polymer films. Blacklock, Oupicky, *et al.*[108] used disulfide-stabilized dendrimer polycations to construct thin films with DNA on the surface of stainless steel mesh. NIH-3T3 and SMC cells readily attached to the polymer-coated meshes, and their subsequent proliferation showed no signs of the biomaterials' cytotoxicity. *In vitro* sustained release of DNA and successful transfection of cells cultured on the thin films was observed for at least 8 days. Using a plasmid encoding a protein which is secreted by cells allowed ascertaining efficiency of transfection *in vivo via* monitoring the concentration of reporter protein in the blood. The level of reporter protein peaked at day 5 which, together with a half-life of this protein in blood of ~17 h, suggested a sustained continuous transgene expression, which was mediated by the subcutaneously implanted surface with deposited DNA-containing multilayers. In another report[109] plasmid DNA and chitosan were sequentially deposited on titanium surfaces to investigate surface-mediated delivery of a gene encoding for BMP-2 and a resulting differentiation of mesenchymal bone marrow stem cells into osteoblasts. Deposited films exhibited a burst release followed by a sustained DNA release profile for over 160 h, and the deliverable dose was effectively controlled by the number of polymer layers deposited. Expression of reporter genes by cells grown on the polymer-coated surfaces was pronounced and higher than that achieved using lipofectamine 2000. Furthermore, while lipofectamine-transfected cells expressed BMP-2 at day 3 but not day 7, surface-mediated transfection resulted in production of BMP-2 even at day 7. Differentiation of cultured MSC into osteoblasts was ascertained through the activity of alkaline phosphatase at days 7 and 14, and the latter was significantly higher for the surface-mediated delivery of DNA as compared to that achieved using lipofectamine 2000 transfection. Further, osteocantin, a marker indicating late stage differentiation of osteoblasts, was also found to be statistically higher in cells transfected from the surface than that for lipofectamine 2000. We believe that these studies present significant advances for the field of surface-mediated gene delivery and guided stem cell differentiation.

Outlook. Overall, we believe that examples presented in this review illustrate that despite a very young history, surface-mediated drug delivery using polymer thin films has already demonstrated highly successful approaches and developed innovative and promising techniques. We envision that one of the plausible developments in the field will relate to concurrent delivery of multiple, structurally and functionally differed therapeutic candidates mediated from the same surface. By design, multilayered polymer films are well fit to accommodate this, and indeed the concept has already been briefly considered in some of the instances discussed in this paper. To give a further successful example, a DNA plasmid for transfection and an α-MSH peptide, a melanocyte stimulating hormone, were incorporated and shown effective within the same multilayered film.[110] The peptide molecule was incorporated as a conjugate with PGA, whereas DNA was adsorbed onto the film surface in a polyelectrolyte complex with PEI. In contact with the functionalized multilayers, B16-F1 cells were producing a greater amount of melanin, that is, were positively sensing the presence of the hormone peptide in the multilayers, and these films were also effective in eliciting surface-mediated gene transfer. In other words, this report demonstrates the possibility to deliver two different drug candidates with different modes of action mediated from the same surface coating. This concept was also well demonstrated in a very recent example which also presents polymer thin films investigated on a largely unexplored territory, namely, transcutaneous vaccination.[111] To this end, degradable multilayered polymer films were deposited onto a skin-adhesive patch using poly(β-aminoesters) as degradable polycations, as described in several instances above, ovalbumin as a common model vaccine, and CpG oligonucleotides as adjuvants. The protein was effectively incorporated into multilayers and upon drying and rehydration was released in a nonaggregated form, making this technology attractive for practical applications. Vaccine protein released by the thin films in contact with skin was permeating into skin, upon which it was internalized by the antigen presenting Langerhans cells, and transported into the lymph nodes, during which dendritic cells underwent maturation, all of which is required for an effective immune response. This study further demonstrates the flexibility and multifunctionality of multilayered thin polymer films through incorporation of the antigen and adjuvant molecules in the same film at varied depths to achieve optimized time-delayed delivery of the two components. We believe this work significantly broadens the horizons in the use of polymer thin films, specifically for vaccination and transdermal delivery of therapeutics.

A brief look through the literature cited in this review demonstrates a significant increase in successful examples of polymer thin films applied for surface-mediated drug delivery, specifically during the past 2−3 years. We therefore expect a further increase in the number of these reports and a growing interest to this area of fundamental and applied research from a broader scientific community, and we hope that this review will contribute to these developments.

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