TISSUE ENGINEERING: Part B
Volume 17, Number 2, 2011

DOI: 10.1089/ten.teb.2010.0548

Polyelectrolyte Multilayers in Tissue Engineering

Christopher J. Detzel, Ph.D.,[1,*] Adam L. Larkin, B.S.,[1,*] and Padmavathy Rajagopalan, Ph.D.[1,2]

The layer-by-layer assembly of sequentially adsorbed, alternating polyelectrolytes has become increasingly important over the past two decades. The ease and versatility in assembling polyelectrolyte multilayers (PEMs) has resulted in numerous wide ranging applications of these materials. More recently, PEMs are being used in biological applications ranging from biomaterials, tissue engineering, regenerative medicine, and drug delivery. The ability to manipulate the chemical, physical, surface, and topographical properties of these multilayer architectures by simply changing the pH, ionic strength, thickness, and postassembly modifications render them highly suitable to probe the effects of external stimuli on cellular responsiveness. In the field of regenerative medicine, the ability to sequester growth factors and to tether peptides to PEMs has been exploited to direct the lineage of progenitor cells and to subsequently maintain a desired phenotype. Additional novel applications include the use of PEMs in the assembly of three-dimensional layered architectures and as coatings for individual cells to deliver tunable payloads of drugs or bioactive molecules. This review focuses on literature related to the modulation of chemical and physical properties of PEMs for tissue engineering applications and recent research efforts in maintaining and directing cellular phenotype in stem cell differentiation.

Introduction

Layer-by-layer (LbL) assembly of polyelectrolytes (PEs) is a method for preparing highly tunable thin film polymer coatings. Such coatings or films are denoted henceforth as polyelectrolyte multilayers (PEMs).[1–3] PEMs self-assemble due to electrostatic interactions between sequentially deposited, alternately charged PEs.[2,4–6] The chemical composition and concentration of the individual PEs direct the self-assembly process of PEMs. Since electrostatic interactions enable the links between PEs, multilayer properties can be modulated through the adjustment of deposition conditions. Typically, parameters that are varied are the pH of the PE solution,[4,7–12] ionic strength,[7,12] the number of layers,[13,14] the order in which the layers are deposited,[15] and post-assembly modifications.[10,11,16–19] A commonly used post-modification process is cross-linking, which further influences film morphology, thickness, and structure.[16,17,20] Virtually any combination of cationic and anionic PEs can be used to assemble PEMs with highly tunable properties applicable in a wide range of applications. Such applications include microelectronics,[11] nanofluidics,[21,22] virucidal coatings,[23] drug delivery,[24,25] and tissue engineering.[26,27] In this review, applications of PEMs in tissue engineering will be discussed.

PEMs have been assembled from synthetic as well as naturally occurring polymers.[1–3,28–30] Synthetic PEMs have been assembled from cationic PEs such as poly (allylamine hydrochloride) (PAH) and poly (diallyldimethylammonium chloride) (PDAC) and anionic PEs such as poly (acrylic acid) (PAA) and sulfonated polystyrene (SPS).[8,29,31,32] PEMs have also been fabricated from naturally occurring PEs such as, polypeptides, polysaccharides, DNA, and proteins.[26,31,33–37] One of the earliest reports of PEMs derived from a combination of synthetic and natural PEs was by Lvov *et al.* where ultrathin films comprised of DNA and PAH were assembled for applications in ecological sensors.[1] More recently, Dubas and Schlenoff explored combinations of poly(styrene) and PDAC to systematically determine the factors governing LbL assembly such as thickness, ionic concentration, and pH.[12] Elbert *et al.* formed PEMs comprised of poly (L-lysine) (PLL) and alginate (ALG) to render the surfaces biologically inert and thereby block the adhesion of human fibroblast cells.[33] Berg *et al.* assembled PAA and arginine-glycine-aspartic acid (RGD)-modified PAH multilayers to promote the cellular adhesion of murine wild-type NT6 fibroblasts and to determine the effect of RGD-PAH spatial configuration (i.e., RGD-PAH line widths ranging from 10 to 50 μm) on cytoskeletal protein organization.[38] Due to a wide range of PEs available to assemble multilayer films, as well the ability to tailor their properties by varying the deposition conditions, PEMs are being incorporated into a variety of biological applications. Their uses include coatings to either promote or prevent cell adhesion, and more importantly in directing or maintaining

[1]Department of Chemical Engineering and [2]ICTAS Center for Systems Biology of Engineered Tissues, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
*These two joint first authors made equal contributions.

cellular phenotype.[29] In addition to PEs, multilayer films have been assembled from DNA strands using complimentary base pairing,[39] click chemistry utilizing covalent bonding,[40] and hydrogen bonding to create degradable multilayers for drug release applications.[41] The remainder of this review will focus on the recent literature regarding LbL assembly of PEs and their applications in the field of tissue engineering.

PEMs have garnered immense interest in the field of biomedical engineering to render implantable material surfaces more biomimetic.[42] Largely due to their tunable nature, PEMs have had a significant impact in nearly every aspect of biomaterial design and tissue engineering.[10,18,22,24,26,27,30,31,38,42–55] PEMs have been used to modulate protein adsorption,[33] promote cell adhesion,[16,29,34,38,55–57] or regulate the inflammatory response.[58–60] In addition, PEM coatings have the potential to mimic the complex extracellular matrix found *in vivo*. To recapitulate the *in vivo* environment, studies have been conducted to tailor the surface topography, chemical composition, mechanical properties, and the degradation of PEMs.[61–63] In subsequent sections, the effect of chemical and mechanical properties of PEMs and their impact on tissue engineering, cell adhesion, maintaining, and, more importantly, directing cellular phenotype will be discussed. Other novel applications of PEMs in tissue engineering such as drug delivery and drug reservoir construction for controlled release will also be reviewed.

Tailoring the Mechanical and Chemical Properties of PEMs for Cellular Applications

The adhesion of cells *in vitro* and their subsequent performance is often dependent on the physical properties of the underlying substrate.[52,62] The mechanical properties of a PEM can be varied by changing the thickness, the pH of the solutions used to assemble the PEM, and through post-assembly modifications. In the following sections, we will review the approaches to tailor the mechanical and chemical properties to optimize cellular response.

Assembly conditions modulate the mechanical properties of PEMs

Various factors such as the assembly conditions and the number of bilayers in the PEM contribute to the diversity in mechanical properties. In a recent study, the response of smooth muscle cells (SMCs) was directly linked to the Young's modulus of the multilayer on which the cells were cultured.[16] Richert *et al.* demonstrated that the number of layers within the self-assembled film play a dominant role in SMC response.[16] PEMs were assembled using PLL and hyaluronic acid (HA) as the cationic and anionic PEs, respectively. Increasing the PLL/HA bilayer number from 20 to 60, directly correlated with increasing thickness from 3 to 15 μm, respectively. However, the Young's modulus was inversely related to bilayer number, with values of 90 and 40 kPa observed for 20 and 60 bilayers, respectively. Thicker PEMs were less rigid due to a higher degree of hydration.[5,64] When SMCs were cultured on the PLL/HA multilayer, projected cell areas were observed to be $\sim 1100\,\mu m^2$ on substrates comprised of 20 bilayers, compared to $800\,\mu m^2$ for a substrate coated with 60 bilayers.[16] In another study by the same group, the adhesion of human chondrosarcoma cells increased with decreased PLL/poly (L-glutamic acid) (PLGA) PEM thickness.[56] Although the thickness of a PEM can affect cellular adhesion and spreading, it appears that swelling and mechanical properties such as Young's modulus may play a more significant role in cellular response.[56]

A self-assembled PEM can grow in a linear[2] or exponential manner[33] (Fig. 1). Linear growth typically occurs when the PEs are highly charged and do not diffuse freely throughout the PEM.[2] Highly charged PEs exhibiting linear growth include cationic PAH and anionic SPS.[2] On the other hand, exponential growth occurs in the presence of weak PEs (PLL, ALG, and PLGA), characterized by diffusion, and hydrogen bonding.[13,33,34,65] In a few cases, linear growth in the presence of weak PEs has also been observed.[66] Exponential PEM growth is largely due to the diffusion of at least one PE through the film,[34,65] resulting in an exponentially increasing thickness, as additional bilayers are added to the film.[66,67] Picart *et al.* attributed the exponential growth observed in PLL/HA PEMs to the diffusion of PLL chains into the interior of the PEM when in contact with the PLL solution.[28] Upon immersion in a HA solution, the PLL chains diffused to the surface and interacted with the highly charged HA chains, resulting in large PE aggregates at the surface resulting in an exponential increase in PEM thickness as subsequent bilayers were deposited.[28] Researchers have exploited the two different modes of growth within the same PEM to create distinct regions. For example, the combination of linear and exponential growth modes can be used to create compartments where the PEs diffuse freely within an exponentially growing region of bilayers and tightly bound in areas of linear growth.[68] Taken together, this methodology exhibits enormous potential for applications in drug delivery, specifically as drug reservoir compartments.[68] Multicompartment PEMs will be discussed later in this review.

The height of a PEM is also dependent on the pH of the individual PE solutions and their ionic strength.[4,5,12,29,55] Polymers can be assembled that exhibit thick, loopy structures at lower charge density or thin, rod-like confirmations at a higher charge density.[8] Mendelsohn *et al.* tuned the adhesion of murine NR6WT fibroblasts on PEMs comprised of PAH and PAA or PDAC and SPS by modulating the pH of the PE solution.[29] PEMs comprised of PAH and PAA, as-

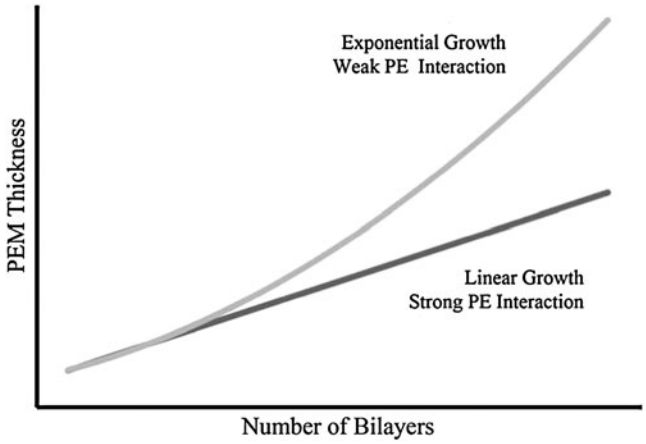

FIG. 1. Relationship between thickness of a PEM and either linear or exponential growth. PEM, polyelectrolyte multilayers; PE, polyelectrolyte.

sembled at pH 6.0, and exhibited excellent cell adhesion compared to PEMs assembled at pH 2.0. When PEMs were assembled at higher values of pH, the multilayers were more rigid in contrast to films assembled at pH 2.0. The observed decrease in cell adhesion was attributed to a higher extent of hydration.[29] Thompson *et al.* assembled identical PEMs to directly relate stiffness, which was modulated by pH, to microvascular endothelial cell adhesion.[55] Multilayers of PAH and PAA assembled at pH 6.5 exhibited a Young's modulus of 150 MPa. On day 6 in culture, the cell densities were found to be $\sim$100 cells/mm^2.[55] However, when the multilayer was assembled at pH 2.0, the Young's modulus was found to be 200 kPa and the cell density on day 6 was $\sim$20 cells/mm^2. These trends suggested that less rigid multilayers may result in lower rates of proliferation.[55] In separate studies, similar properties were obtained by simply changing the ionic concentration of the PE solutions.[7,12] Typically PEMs are assembled under specific conditions, for example, pH or ionic concentration, to obtain a desired set of properties. However, post-assembly modifications may result in changes in the PEM thickness, stiffness, or porosity.[10,29]

Effects of PEM crosslinking on cell adhesion

Postassembly chemical crosslinking is a method to further modulate mechanical properties. For PEMs with the appropriate functional chemistry, such as amine and/or carboxyl groups, water-based covalent chemical crosslink chemistries have been investigated.[16,32,69–71] Carbodiimide crosslinkers such as 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (commonly referred to as EDC) are crosslinkers used to couple carboxyl and amine groups leading to the formation of amide bonds.[70,72–74] In a recent study, the Young's modulus was varied for PLL/HA PEMs to modulate myoblast adhesion, differentiation, and myotube formation using EDC concentrations ranging from 5 to 100 mg/mL.[57] Increasing the concentration of EDC was found to be directly proportional to the mechanical stiffness of the PEM. Myoblast adhesion increased with higher crosslinker concentration and well-defined focal adhesions were observed only for the stiffest PEMs along with well-organized f-actin fibers. The morphology of the myotubes varied significantly with thinner, more elongated tubes forming only on the stiffest PEMs (Young's modulus of 400 kPa).[57] It is also possible to crosslink PEMs during LbL assembly as demonstrated by Croll *et al.* using EDC.[75] In this study, EDC and N-hydroxysuccinimide (NHS) were added to the HA solution before LbL assembly. PEMs cross-linked by EDC did not degrade in phosphate-buffered saline, whereas those assembled without the crosslinker rapidly degraded. These stable hydrated PEMs were used to prevent protein and cellular adhesion.[75]

Glutaraldehyde (GA) is another common chemical used to crosslink PEMs.[76–78] Tong *et al.* crosslinked PEM microcapsules comprised of PAH and SPS to promote stability for potential applications in drug delivery. GA crosslinking resulted in a 2.3-fold increase in Young's modulus, from 290 to 680 MPa.[79] The critical pressure, upon which microcapsule collapse occurred, doubled upon GA crosslinking for 1 h.[79] More recently, GA crosslinking was utilized to improve the stability as well as to tune the Young's modulus of free-standing HA and chitosan (CHI) PEMs.[18] Upon exposure to GA, the stability of the films in aqueous solutions increased to >90% weight retention over a period of 7 days. However, uncrosslinked films deteriorated in aqueous solutions within 5 min.[18] The Young's modulus increased from 90 MPa for uncrosslinked PEMs to 310 and 480 MPa for PEMs crosslinked with GA exposure for 1 and 2 min respectively.[18] BALB/c 3T3 fibroblasts colonized the entire surface of the crosslinked PEMs within 6 days and demonstrated well-defined actin cytoskeletal structure.[18]

In addition to chemical crosslinkers, other covalent methods can also be used. Recently, Moussallem *et al.* thermally crosslinked PAH and PAA PEMs to form covalent amide crosslinks.[20] The hydrated Young's modulus increased from 6 MPa to 8 GPa for uncrosslinked and crosslinked PEMs, respectively. A7r5 rat aortic SMCs cultured on uncrosslinked PEMs (6 MPa) expressed phenotypic markers, such as vimentin and nonmuscle myosin heavy chain IIB,[20] whereas SMCs expressed markers of a contractile phenotype such as calponin and smooth muscle α-actin when cultured on the 8 GPa crosslinked PEMs.[20] For most cell types, increased substrate stiffness promotes cellular adhesion.[16,29,38,53,55,56,62,69] The range of mechanical properties in PEMs due to assembly conditions can be used to probe cellular mechanics as well as promote cellular functions for tissue engineering (Table 1).

PEM surface composition

The surface composition of a PEM film can drastically impact cellular adhesion.[61] This feature can be altered by varying the final PE deposited during the LbL assembly.[34,55,56,80] Richert *et al.* observed that PEMs terminating in PLL demonstrated higher human chondrosarcoma adhesion forces, $\sim$80 nN, in comparison to PLGA-terminated PEMs, where an eightfold lower adhesion force was observed.[56] More recently, Wittmer *et al.* assembled PLL and dextran sulfate PEMs.[80] *In situ* mass adsorption measurements with a quartz crystal microbalance with dissipation showed a 40% increase in fibronectin adsorption on PLL-terminated PEMs compared to dextran sulfate-terminated multilayers. Further, significant spreading of human umbilical vein endothelial cells was observed only on the PLL-terminated PEM, due to the increased fibronectin adsorption and terminal PLL layer.[80] However, certain polycations have displayed cytotoxic effects. For example, Brunot *et al.* determined the cytotoxicity of poly (ethyleneimine) (PEI) used as a base layer for PEMs and as a PE coating.[81] PEI demonstrated potential cytotoxicity for both human periodontal fibroblasts as well as MG63 osteoblast-like cells.[81] Additionally, Fischer *et al.* investigated the cytotoxicity of polycation solutions such as PEI, PLL, and PDAC.[82] The MTT assays for L929 mouse fibroblasts indicated that PEI was more cytotoxic compared to PLL and PDAC.[82] Together, these results demonstrate that choosing a noncytotoxic polyion is a critical design parameter when assembling PEMs for biological applications.

Surface composition does not always significantly impact cellular adhesion. Thompson *et al.* observed that terminal layer had no statistical significance when PEMs were sufficiently stiff to support cellular adhesion.[55] When compliant PAH and PAA multilayers with an approximate Young's modulus of 200 kPa were used as substrates for microvascular endothelial cells, the terminal layer (comprised of either PAA or PAH) had no statistically significant effect on cell density on day 7.[55] Similarly, chondrosarcoma adhesion on crosslinked PLL- and HA-terminated PEMs did not vary, with both substrates

Table 1. Examples of Parameters Varied in Polyelectrolyte Multilayer Assembly for Cell Culture Applications

Cationic PE/anionic PE	*PEM parameter varied*	*PEM property affected*	*Cell types cultured*	*References*
PAH/PAA, PLL/PLGA, PAH/PMA	Assembly pH	Thickness or swelling	NR6WT fibroblasts, HCS-2/8 human chondrosarcoma cells	8,29,73,142
PAH/PAA, PAH/SPS	Assembly pH	Stiffness	MVEC	55,143,144
PAH/PAA-modified RGD	Assembly pH	Surface composition	NR6WT fibroblasts, osteoblasts	38,99,145,146
PAH/PAA	Assembly pH	Surface topography	Human corneal epithelial cells	10,11,44
PLL/PLGA, PLL/alginate	Number of layers	Thickness	Human fibroblasts, fetal liver/stem progenitor cells	33,120
(PEI or PLL or PAH)/ (SPS or PLGA), PLL/PLGA, PLL/HA, PLL/(PLGA or PAH or SPS)	Order in which the PEs were assembled	Terminating PE Layer	Periodontal ligament cells, HCS-2/8 human chondrosarcoma cells, primary human monocytes	54,142,147,148
PLL/HA	Chemical crosslinker (EDC)	Stiffness	Chondrocytes, C2C12 myoblasts, SMCs	16,52,57,69,149
PEI/PAA+MWNT, PAH/SPS, HA/chitosan	Chemical crosslinker (GA)	Stiffness	BALB/c 3T3 fibroblasts	18,78,79
PLL/vinylbenzyl grafted-HA, PAH/PAA	Other modes of crosslinking (Heat, UV)	Stiffness	C2C12 myoblasts, A7r5 SMCs	17,20

EDC, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride; GA, glutaraldehyde; HA, hyaluronic acid; MVEC, microvascular endothelial cell; MWNT, multiwall carbon nanotube; PAA, poly (acrylic acid); PAH, poly (allylamine hydrochloride); PE, polyelectrolyte; PEI, poly (ethyleneimine); PEMs, polyelectrolyte multilayers; PLGA, poly (L-glutamic acid); PLL, poly (L-lysine); RGD, arginine-glycine-aspartic acid; SMCs, smooth muscle cells; SPS, sulfonated polystyrene; PMA, poly(methacrylic acid); UV, ultraviolet.

almost entirely colonized by day 6.[83] These studies suggest that there is a subtle interplay between cellular adhesion and chemical and physical properties of PEMs.

PEM surface morphology

Topographical cues play an important role *in vivo*, since cells are exposed to environments ranging from the micro- to the nanoscale.[61,63] The ability to control PEM surface topography is extremely important in tissue engineering. Basement membranes *in vivo* typically exhibit porosities ranging from the nano- to the microscale to enable the diffusion of gases and nutrients to the cells.[84–86] When PAA and PAH multilayers were exposed to an acidic aqueous solution (pH 2.5), microporous PEMs were obtained (Fig. 2). The transition from a nonporous to porous state was attributed to polymer cleavage and reorganization.[10] The characteristic pore length was 100–500 nm after 60 s exposure to an acidic solution (pH 2.4). Upon treatment at neutral pH for >1 h, pore size diminished to ~50–200 nm. By heating PEMs above 200°C, amidization occurred and created inter- and intrachain crosslinks, and the films were found to be stable under ambient conditions up to 18 months.[10] In another study, Hiller *et al.* used a similar approach to introduce nanoscale pores in PAH/PAA films.[11] By reducing the pH of the acidic solution to 1.8, pore sizes were reduced to 15–80 nm.[11] In addition to lowering the pH, salts such as NaCl and $MgCl_2$ were added at low concentrations to promote structural rearrangements.[11,87] Recently, nano- and microporous PEMs were used to mimic the basement membrane in the cornea (typical porosity ranging from 20 to 200 nm).[44,84,85] PAA and PAH multilayers exhibiting pore sizes ranging from 50 to 240 nm were used to culture human corneal epithelial cells (HCECs).[44] HCECs cultured on nanoporous PEMs exhibited two-fold higher migration speeds in comparison to microporous films, 30 and 15 μm/h, respectively.[44] In addition, HCECs exhibited well-defined actin cytoskeletal structures and vinculin focal adhesions only on the nanoporous substrates.[44] These examples demonstrate that by simply varying the surface topography of a PEM, cellular response can be controlled.[44,88]

Applications of PEMs in Tissue Engineering

Maintenance of cellular phenotype is paramount in any tissue engineering application. The ability to measure *in vitro* cellular responses to conditions that recapitulate the environment *in vivo* can provide comprehensive insights into the behavior of implanted biomaterials. PEMs are often designed with finely tuned bulk properties like Young's modulus and degradability, in addition, such films can be further modified to maintain or induce specific phenotypic characteristics.[89,90] In the following sections, the use of PEMs in the fields of regenerative medicine and stem cell differentiation will be highlighted.

Maintaining cellular phenotype using PEMs

The cellular adhesion and phenotypic properties of SaOS-2 (human osteoblast-like cells) and human periodontal ligament cells on multilayers derived from various PEs were investigated by Tryoen-Toth *et al.*[54] In this study, anionic SPS

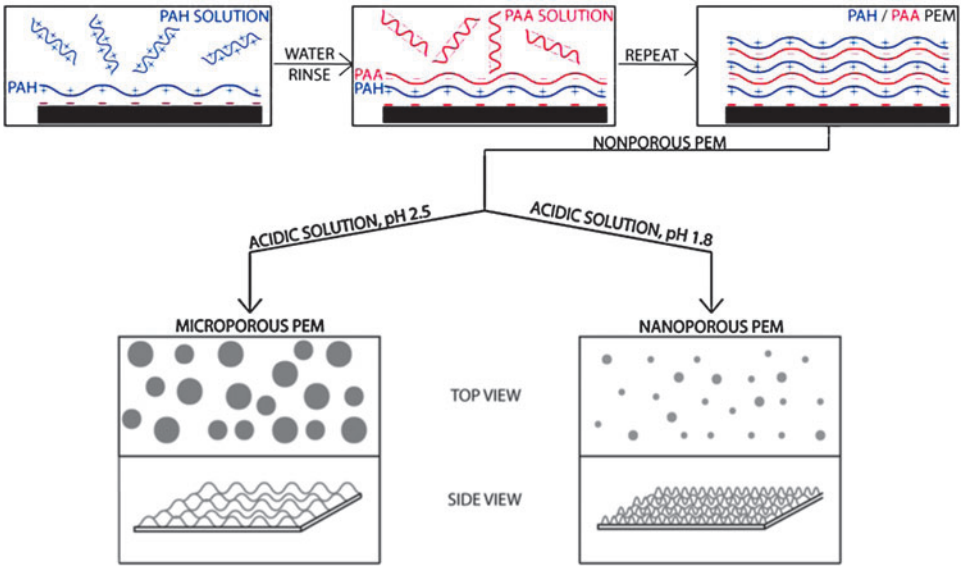

FIG. 2. Schematic for creating non-, micro-, and nanoporous PAH/PAA PEMs. PAA, poly (acrylic acid); PAH, poly (allylamine hydrochloride). Color images available online at www.liebertonline.com/teb.

and PLGA were utilized in combination with cationic PEI, PAH, and PLL to construct a wide range of PEMs. In these studies, the terminal PE layer drastically affected cellular adhesion, but more importantly, this feature had a significant effect on influencing cellular phenotype. Measurement of alkaline phosphatase mRNA levels indicated the terminal PE layer must be negatively charged to maintain osteoblast phenotype in both cell lines. Negatively charged PGA and SPS terminating films resulted in stable expression levels, unlike cationic PEI and PAH terminal films.[54] Interestingly, it was also shown that these cells interacted only with the terminal PE layer in the substrate. For example, PGA deposited directly on top of PAH exhibited positive phenotypic effects by the osteoblast-like cells, whereas reversal of these two layers resulted in negative phenotypic expression.[54] These results suggest the terminal PEM layer is a dominant factor in biocompatibility and must be carefully considered based on desired cellular interactions for a particular application. Many additional studies have investigated adhesion, proliferation, spreading, and phenotypic expression profiles of many different cell types on PEMs constructed from combinations of PEs (Table 2).

In addition to facilitating phenotypic maintenance, it is important for PEMs to stimulate cellular proliferation. Various growth factors can stimulate proliferation, and to this end their incorporation and controlled release from PEMs may be necessary for regenerative medicine. A common problem with growth factor administration is uncontrolled

Table 2. Examples of Polyelectrolyte Multilayers Used in Tissue Engineering Applications

Research objective	*Cationic/anionic PE*	*Cell type*	*Significant finding*	*References*
Cell interaction	PLL/HA CHI/HA PLL/PGA	Chondrocytes, chondrosarcoma	Crosslinking increased Young's modulus and cell adhesion, and spreading decreased enzymatic degradation	149–151
	PEI/PAA SPS/PAH CHI/HA Amine Mod HA/HA	Fibroblasts	Adhesion, proliferation, and cell morphology	152,153
	SPS/PAH CHI/HA PAA/PAH	Endothelial cells	Adhesion, spreading, and viability	154–157
Delivery of bioactive molecules	PLL/RNA PLL/PLGA CHI/HA PLL/HA	Osteoblasts, COS-1; HeLa; HEK-293	DNA or RNA incorporation to inhibit gene expression or as transfection vehicle	158–161
	PLL/PGA PLL/HA PAH/PAA	Cancer cell lines (CHO, melanoma, HT29); macrophages; fibroblasts	Embedded drugs, growth factors, or signaling molecules for cellular response modulation	162–167

CHI, chitosan.

release leading to high initial concentrations followed by periods of rapid clearance, thus limiting possible therapeutic effects.[91,92] The release of fibroblast growth factor 2 (FGF-2) has been controlled with the construction of poly (β-aminoester)s/heparin (HEP)/FGF-2/HEP tetralayers deposited on top of PEI/SPS multilayers.[43] This approach was similar to another study using tetralayers for controlled hydrophobic drug and polysaccharide release.[93] The incorporation of the poly (β-aminoester) polymer into the tetramer was to prevent the localized degradation of the PEM due to cellular interactions. Ester bonds in the poly (β-aminoester) were hydrolytically cleaved during cellular degradation.[93] The incorporation of methylene units to increase hydrophobicity resulted in decreased degradation. The incorporation of HEP into tetralayers was necessary due to its known sequestration of FGF-2,[94] thereby inhibiting the diffusion of free FGF-2 between tetralayers. Thus, the tetralayer method of encapsulating FGF-2 proved to be very useful since the protein from a single layer had to be completely released before release from the underlying layers. The bioactivity of the released protein was verified through its exposure to preosteoblasts and by monitoring their proliferation.[43] Information on additional studies investigating the delivery of bioactive molecules using PEMs is provided in Table 2, further supporting the versatility of PEMs in stimulating phenotypic maintenance.

When short functional peptide sequences are tethered to a substrate, they can modulate a wide range of responses. For instance, the tri-peptide sequence, RGD, has been extensively utilized to promote cell adhesion and migration.[95,96] This approach has been particularly successful for cells that preferentially adhere to fibronectin.[19,97] A comprehensive review of the chemistry involved in RGD and other peptide conjugation to synthetic polymers has been previously been reported.[98] To demonstrate the efficacy of PEMs functionalized with short peptide sequences, Tsai *et al.* constructed PAH/PAA PEMs comprised of five bilayers, with a terminal RGD-PAH layer.[99] In this study, the incorporation of RGD stimulated osteoblast adhesion, proliferation, and phenotype.[99] The assembly of the multilayer was conducted at pH of 2 and 6.5. At lower pH, the anionic PE was incorporated at increased nonstoichiometric ratios to compensate for the fully ionized cationic PE (~70% PAA, 30% PAH at pH 2.0).[99,100] Consequently, when the terminal RGD-PAH layer was deposited, the concentration of RGD molecules at the surface increased fivefold at pH 2.0.[99] Despite the increased RGD surface concentration, osteoblast adhesion and proliferation were unaffected since the concentration of RGD exceeded 0.6 pmol/cm^2.[99] This value has been reported to be the threshold concentration, necessary to stimulate osteoblast adhesion and spreading.[101] However, at pH 2.0 a threefold increase in the deposition of calcium by osteoblasts was observed. Although the exact mechanism of increased calcium deposition in this study remained unknown, the authors hypothesized that decreased PEM mechanical stiffness, 100 MPa at pH = 6.5–1 MPa at pH = 2.0,[55] or RGD conformational changes due to pH changes may be responsible for the observed increased calcium deposition. This study serves as a good example on the ability to elicit a desired phenotypic response by simply varying the assembly conditions of a PEM.

Many studies have focused on maintaining cellular phenotype through direct cellular interaction with PEMs. The adhesion of hepatocytes and maintaining their phenotype is one such example.[96] In general, primary hepatocytes cultured *in vitro* as monolayers tend to rapidly de-differentiate. They lose their ability to produce urea (indicative of liver specific carbohydrate, lipid, and amino acid metabolism) and albumin (representing hepatic protein production). PAA/PAH multilayers have been used to culture hepatocytes and these substrates appear to elicit increased urea and albumin production in comparison to hepatocytes cultured on a collagen surface.[102] Similarly, Wittmer *et al.* have demonstrated that both PLL/ALG and PLL/PLGA PEMs facilitate adult rat hepatocyte adhesion and increased albumin production when compared to cells cultured on a collagen surface.[103] Further, in both reports, the adsorption of collagen on the terminal PE layer enhanced hepatocyte adhesion and phenotypic functions.[102,103] Hepatocytes are generally stable when cultured between two collagen gels (collagen sandwich).[104,105] When these cells are cocultured with nonparenchymal hepatic cells, their function can be further enhanced.[48,106–108] Studies by Kidambi *et al.* have shown that hepatocyte and fibroblast attachment can be spatially controlled on patterned PDAC/SPS PEMs with superior performance.[47,48]

More recently, three-dimensional (3D), layered liver mimics have been designed using PEMs.[26,27] In these 3D tissue mimics, hepatocytes were seeded on a collagen gel and a HA-CHI PEM was assembled above live cells. Next, liver sinusoidal endothelial cells (LSECs) were seeded above the PEM-coated hepatocytes.[27] In these cultures, the PEM served as a synthetic substitute for the Space of Disse, the interface between hepatocytes and LSECs *in vivo*, thus using the PEM to create a physiologically relevant 3D culture system. In these cultures, the physical properties of the PEM, the number of layers, and the relative concentrations of each cell type affected hepatocyte-specific functions.[27] The shear modulus of the PEM within the 3D liver mimic was ~100 kPa, a value similar to that previously shown to maintain hepatic phenotype.[109] Furthermore, the activity of CYP1A1/2, a cytochrome P450 enzyme involved in the metabolism of cyclic aromatic compounds was increased 16-fold over conventional monolayer cultures.[27] These results suggest that PEMs can serve as the interface between cell layers and used to assemble a wide range of stratified 3D tissue mimics.

Directing cellular differentiation using PEMs

A growing body of work is emerging in which PEMs are being tuned to direct phenotypic differentiation, particularly progenitor cells, toward specific lineages. Directing the lineage of stem cells is dependent upon complex interactions between soluble factors, the extracellular matrix, intra- and intercellular communications, and biophysical stimuli.[110–113] Although soluble growth factors are critical to induce directed stem cell differentiation, Engler *et al.* recently showed that mesenchymal stem cells (MSCs) could be directed to either neuronal, muscle, or osteogenic lineages by simply modulating substrate elasticity from <1, to 8–17, to 25–40 kPa, respectively.[53] Since PEMs exhibit mechanical properties in this range, the results from this study can be used in directing phenotype.[16,17,57] PEMs composed of alternating layers of PAH and SPS have been shown to stimulate the differentiation of endothelial progenitor cells.[114–116] En-

dothelial progenitor cells are a specialized type of progenitor cells with a limited number of potential lineages. However, when these cells were cultured on PEMs in the presence of culture medium containing growth factors, such as vascular endothelial growth factor and basic FGF, the differentiation into endothelial phenotype was observed.[114,117] PEMs derived from PEI and SPS with embedded carbon nanotubes enabled neuronal progenitor cell differentiation into the three main classes of neural cells.[118] These studies show PEMs can aid in directing the differentiation of progenitor cells.

Liu *et al.* studied the adhesion and differentiation of MSCs when cultured on PEMs comprising of alternating layers of CHI with either gelatin (GEL), HA, or HEP.[119] In this study, MSC adhesion increased for the following PEM combinations: GEL/CHI, HA/CHI, and HEP/CHI.[119] It was further reported that MSC spreading was inversely proportional to cell adhesion. Although cell spreading could be modulated through the careful selection of PEs, the authors concluded a high degree of cell spreading, observed on GEL/CHI, was not indicative of MSC phenotype. Studies by Semenov *et al.* further validated these findings by culturing MSCs on PEMs exhibiting a range of mechanical properties showing chondrogenic and osteogenic lineage differentiation was dependent on the Young's modulus.[6]

One of the most critical aspects of stem cell research is selecting and isolating a pure population of cells. Fluorescence-activated cell sorting is a powerful technique used in the isolation of populations of pure cells. However, due to the inherent complexity of this technique, and in obtaining viable, antibody-free cells, this technique presents limitations.[120–122] A very interesting application of PEMs was reported by Tsai *et al.* where fetal liver stem/progenitor cells could be selected from a gross tissue digest using PLGA-terminating PLL/PLGA multilayers. Further, these substrates stimulated cell proliferation over time.[120] The development of such a cell selection method could yield a more precise and less expensive approach to selectively isolate pure cell populations.

Another novel use of PEMs in cell culture was to culture MSCs on thermo-responsive multilayers made from SPS and PAH, copolymerized with N-isopropylacrylamide.[123] The use of thermo-responsive PEMs enabled MSC proliferation and passaging without enzymatic treatment. This procedure yielded an increased number of MSCs capable of osteogenic and adipogenic lineage differentiation after multiple passages.[123] The combination of these two studies illustrates the application of PEMs for the selection and culture of stem cells in a noninvasive manner.

Multifunctional PEMs for Tissue Engineering

Although advances have been made toward the incorporation of bioactive molecules into PEMs, an unmet challenge continues to be the inclusion of small, uncharged, hydrophobic molecules.[49,124,125] Such compounds account for ~40% of the drugs approved by the Food and Drug Administration.[41] A recent development has been the construction of free-standing multilayer films comprised of block copolymer micelles and PAA.[41] Kim *et al.* demonstrated that the hydrophobic antibacterial drug triclosan,[41] remained active after micelle incorporation and could be successfully released at physiological conditions. It was also shown that the rate of film degradation and drug release could be tailored based through changes in the degree of crosslinking within the PEM. A similar approach to protect and deliver bioactive molecules is their incorporation within polymersomes.[126–128] Polymersomes are bilayer membrane capsules formed through the self-assembly of ampiphilic block copolymers.[129] Biodegradable functionalized polymersomes exhibit potential for site-specific therapeutic delivery through incorporation into PEMs. Further expanding on this idea of drug encapsulation is the construction of capsosomes. Capsosomes are constructed from alternating layers of liposomes and an alternately charged polymer using LbL assembly.[130,131] The multiple liposome layers allow multiple reactions to occur simultaneously while being spatially separated within a single capsosome.[130]

Another novel application of PEMs in drug delivery is the construction of multiple drug reservoirs within a single degradable PEM. Drug reservoirs are constructed by exploiting the characteristic diffusivity associated only with exponentially growing PEMs, not normally observed under linear growth.[68] The physical differences between exponentially and linearly grown PEM films have already been discussed in detail in the section Assembly conditions modulate the mechanical properties of PEMs. Drug reservoirs take advantage of exponentially grown PEMs in which bioactive molecules are incorporated. Due to the higher diffusivity associated with such regions, bioactive molecules can easily diffuse, thereby providing a means for localized delivery at high concentration.[13] A schematic of the layered architecture required to create a PEM film containing multiple drug reservoirs is shown in Figure 3. The construction of such reservoirs requires the presence of impermeable barriers on either side of the exponentially grown PEMs. Impermeable barriers to prevent drug diffusion include linearly grown films,[65] an annealed layer of charged wax particles,[132] or a layer of biodegradable PLGA.[68] Garza *et al.* created two drug reservoirs within a single film in which each reservoir was separated by a layer of PLGA. In this instance the PLGA layer was deposited above the terminal PEM layer using a novel spray deposition technique to provide the biocompatible biodegradable layer separating the reservoirs.[68] The PLGA layers prevented direct cell contact with the drugs in each reservoirs up to 5 days, a length of time which correlated directly with time the cells took to degrade the PEM.[68] It has also been shown that cells do not have to come in

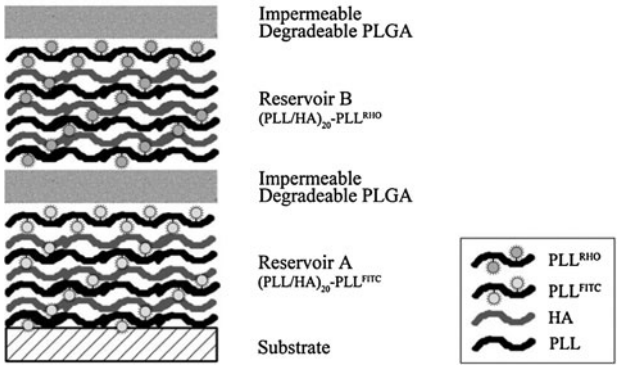

FIG. 3. Schematic of the layered architecture for the design of a multireservoir drug delivery PEM. PLGA, poly (L-glutamic acid); PLL, poly (L-lysine); HA, hyaluronic acid.

direct contact with the reservoirs, but can internalize drugs through localized degradation of the PLGA layer.[133,134]

A final application of PEMs to be discussed in tissue engineering involves living cells functionalized with multilayer patches.[50] This work represents an elegant combination of several PEM applications and techniques outlined in this review. Recent advances in PEM applications have led to the construction of multilayer assemblies encapsulating entire living cells[135–139]; however, the entire surface chemistry of the cell is likely altered affecting cell communication and environmental interactions.[50] In the work by Swiston *et al.* a three-tiered construct was developed where each PEM tier had a specific function: the releasable region, the payload region, and the cell-adhesive region.[50] The three-tiered construct was built from a substrate on which a photolithographic patterning method was used to create the first tier, a layer capable of being detached from the substrate through a combination of pH and thermal conditions. The second tier, the payload region, of the cell patch was engineered as a drug reservoir to contain a range of bioactive molecules. In this case, the payload PEM consisted of alternating fluorescent-PAH and anionic super-paramagnetic nanoparticles. The final tier in the patch was the cell-adhesive region constructed from a HA/CHI PEM. After patch construction, individual cell-patch attachment was observed only for 10 μm patches and cell aggregates were observed on 15 μm patches.[50] After release from the substrate, these cells could be magnetically manipulated due to the presence of the PEM patch. A significant consequence was that the patches did not interfere with the phenotype and physiology of the cell.

Conclusions and Future Possibilities

The tunability of PEMs provides unique opportunities to mimic the complex *in vivo* extracellular matrix environment. Chemical, mechanical, and topographical features can be varied to promote cellular adhesion, differentiation, migration, and gene expression of virtually any cell type. Currently, the majority of PEM applications in tissue engineering are focused on rendering two-dimensional surfaces compatible for biological applications. However, it is well known that providing a 3D environment to cells has the potential to elicit an optimal response.[61] Modifications in the current techniques used to deposit multilayers on two-dimensional substrates will be critical to successfully incorporate PEMs into the design of 3D scaffolds.

An ultimate objective in tissue engineering is to functionalize implantable materials directing cellular adhesion, phenotype, and enhance site-specific regeneration of damaged or diseased tissues. One such instance is the construction of 3D tissue constructs involving multiple cell types. To this point, most PEM-related tissue engineering applications have focused on surface coatings. However, due to the tunable nanoarchitecture of PEMs they can be used in the construction of 3D liver mimics.[27] The PEM must provide a mechanical support for LSEC culture, a physical barrier between hepatocytes and LSECs, but most importantly allow diffusion of signaling molecules to facilitate cell–cell communication and phenotypic maintenance of both cell types. Recent efforts to create free standing PEMs comprised of synthetic and biologically derived PEs[18,140,141] exhibit tremendous potential to be utilized as scaffolds in tissue engineering. An application with much potential in biological therapeutic applications is the coating of individual cells with biocompatible PEMs. Use of PEMs to coat a cell will likely alter its biological activity, but a carefully chosen combination of PEs may result in optimal physiological function.

Finally, the number of tissue engineering application employing PEMs are rapidly expanding and providing useful insights into cellular responses to different combinations of mechanical and chemical signals. In the near future, PEM applications studied *in vitro* for drug delivery and directing cellular phenotype will undoubtedly proceed to *in vivo* applications and clinical trials. Current research and emerging ideas to functionalize surfaces for biological applications will benefit from the unique and highly tunable nature of PEMs as they can be deposited on any surface with a wide range of mechanical and chemical properties.

Acknowledgments

We gratefully acknowledge financial support from the National Science Foundation (DMR-090750, P.R.), the National Institutes of Health (NIDDK-1R21DK077802, P.R.), the Institute for Critical Technology and Applied Sciences (P.R.), and the MILES-NSF IGERT program at Virginia Polytechnic Institute and State University (A.L.L. and P.R.).

Disclosure Statement

No competing financial interests exist.

References

1. Lvov, Y., Decher, G., and Sukhorukov, G. Assembly of thin-films by means of successive deposition of alternate layers of DNA and poly(allylamine). Macromolecules **26,** 5396, 1993.
2. Decher, G. Fuzzy nanoassemblies: toward layered polymeric multicomposites. Science **277,** 1232, 1997.
3. Decher, G., Hong, J.D., and Schmitt, J. Buildup of ultrathin multilayer films by a self-assembly process. 3. Consecutively alternating adsorption of anionic and cationic polyelectrolytes on charged surfaces. Thin Solid Films **210,** 831, 1992.
4. Schlenoff, J.B., and Dubas, S.T. Mechanism of polyelectrolyte multilayer growth: charge overcompensation and distribution. Macromolecules **34,** 592, 2001.
5. Dubas, S.T., and Schlenoff, J.B. Polyelectrolyte multilayers containing a weak polyacid: construction and deconstruction. Macromolecules **34,** 3736, 2001.
6. Semenov, O., Malek, A., Voros, J., and Zisch, A. Human stem cell sheets engineered on polyelectrolyte multilayer coatings for prospective transplantation. Tissue Eng Part A **15,** 714, 2009.
7. Choi, J., and Rubner, M.F. Influence of the degree of ionization on weak polyelectrolyte multilayer assembly. Macromolecules **38,** 116, 2005.
8. Shiratori, S.S., and Rubner, M.F. pH-dependent thickness behavior of sequentially adsorbed layers of weak polyelectrolytes. Macromolecules **33,** 4213, 2000.
9. Sukhorukov, G.B., Donath, E., Davis, S., Lichtenfeld, H., Caruso, F., Popov, V.I., *et al*. Stepwise polyelectrolyte assembly on particle surfaces: a novel approach to colloid design. Polym Adv Technol **9,** 759, 1998.

10. Mendelsohn, J.D., Barrett, C.J., Chan, V.V., Pal, A.J., Mayes, A.M., and Rubner, M.F. Fabrication of microporous thin films from polyelectrolyte multilayers. Langmuir **16,** 5017, 2000.
11. Hiller, J., Mendelsohn, J.D., and Rubner, M.F. Reversibly erasable nanoporous anti-reflection coatings from polyelectrolyte multilayers. Nat Mater **1,** 59, 2002.
12. Dubas, S.T., and Schlenoff, J.B. Factors controlling the growth of polyelectrolyte multilayers. Macromolecules **32,** 8153, 1999.
13. Lavalle, P., Gergely, C., Cuisinier, F.J.G., Decher, G., Schaaf, P., Voegel, J.C., *et al.* Comparison of the structure of polyelectrolyte multilayer films exhibiting a linear and an exponential growth regime: an *in situ* atomic force microscopy study. Macromolecules **35,** 4458, 2002.
14. Caruso, F., Niikura, K., Furlong, D.N., and Okahata, Y. Ultrathin multilayer polyelectrolyte films on gold: construction and thickness determination. Langmuir **13,** 3422, 1997.
15. Lowack, K., and Helm, C.A. Molecular mechanisms controlling the self-assembly process of polyelectrolyte multilayers. Macromolecules **31,** 823, 1998.
16. Richert, L., Engler, A.J., Discher, D.E., and Picart, C. Elasticity of native and cross-linked polyelectrolyte multilayer films. Biomacromolecules **5,** 1908, 2004.
17. Vazquez, C.P., Boudou, T., Dulong, V., Nicolas, C., Picart, C., and Glinel, K. Variation of polyelectrolyte film stiffness by photo-cross-linking: a new way to control cell adhesion. Langmuir **25,** 3556, 2009.
18. Larkin, A.L., Davis, R.M., and Rajagopalan, P. Biocompatible, detachable, and free-standing polyelectrolyte multilayer films. Biomacromolecules **11,** 2788, 2010.
19. Massia, S.P., and Hubbell, J.A. Covalent surface immobilization of Arg-Gly-Asp-containing and Tyr-Ile-Gly-Ser-Arg-containing peptides to obtain well-defined cell-adhesive substrates. Anal Biochem **187,** 292, 1990.
20. Moussallem, M.D., Olenych, S.G., Scott, S.L., Keller, T.C.S., and Schlenoff, J.B. Smooth muscle cell phenotype modulation and contraction on native and cross-linked polyelectrolyte multilayers. Biomacromolecules **10,** 3062, 2009.
21. Makamba, H., Kim, J.H., Lim, K., Park, N., and Hahn, J.H. Surface modification of poly(dimethylsiloxane) microchannels. Electrophoresis **24,** 3607, 2003.
22. DeRocher, J.P., Mao, P., Han, J.Y., Rubner, M.F., and Cohen, R.E. Layer-by-layer assembly of polyelectrolytes in nanofluidic devices. Macromolecules **43,** 2430, 2010.
23. Wong, S.Y., Li, Q., Veselinovic, J., Kim, B.S., Klibanov, A.M., and Hammond, P.T. Bactericidal and virucidal ultrathin films assembled layer by layer from polycationic N-alkylated polyethylenimines and polyanions. Biomaterials **31,** 4079, 2010.
24. Berg, M.C., Zhai, L., Cohen, R.E., and Rubner, M.F. Controlled drug release from porous polyelectrolyte multilayers. Biomacromolecules **7,** 357, 2006.
25. Wood, K.C., Boedicker, J.Q., Lynn, D.M., and Hammon, P.T. Tunable drug release from hydrolytically degradable layer-by-layer thin films. Langmuir **21,** 1603, 2005.
26. Rajagopalan, P., Shen, C.J., Berthiaume, F., Tilles, A.W., Toner, M., and Yarmush, M.L. Polyelectrolyte nanoscaffolds for the design of layered cellular architectures. Tissue Eng **12,** 1553, 2006.
27. Kim, Y., Larkin, A.L., Davis, R.M., and Rajagopalan, P. The design of *in vitro* liver sinusoid mimics using chitosan-hyaluronic acid polyelectrolyte multilayers. Tissue Eng Part A **16,** 2731, 2010.
28. Picart, C., Lavalle, P., Hubert, P., Cuisinier, F.J.G., Decher, G., Schaaf, P., *et al.* Buildup mechanism for poly(L-lysine)/hyaluronic acid films onto a solid surface. Langmuir **17,** 7414, 2001.
29. Mendelsohn, J.D., Yang, S.Y., Hiller, J., Hochbaum, A.I., and Rubner, M.F. Rational design of cytophilic and cytophobic polyelectrolyte multilayer thin films. Biomacromolecules **4,** 96, 2003.
30. Swierczewska, M., Hajicharalambous, C.S., Janorkar, A.V., Megeed, Z., Yarmush, M.L., and Rajagopalan, P. Cellular response to nanoscale elastin-like polypeptide polyelectrolyte multilayers. Acta Biomater **4,** 827, 2008.
31. Thierry, B., Winnik, F.M., Merhi, Y., Silver, J., and Tabrizian, M. Bioactive coatings of endovascular stents based on polyelectrolyte multilayers. Biomacromolecules **4,** 1564, 2003.
32. Zhang, J., Senger, B., Vautier, D., Picart, C., Schaaf, P., Voegel, J.C., *et al.* Natural polyelectrolyte films based on layer-by layer deposition of collagen and hyaluronic acid. Biomaterials **26,** 3353, 2005.
33. Elbert, D.L., Herbert, C.B., and Hubbell, J.A. Thin polymer layers formed by polyelectrolyte multilayer techniques on biological surfaces. Langmuir **15,** 5355, 1999.
34. Richert, L., Lavalle, P., Payan, E., Shu, X.Z., Prestwich, G.D., Stoltz, J.F., *et al.* Layer by layer buildup of polysaccharide films: physical chemistry and cellular adhesion aspects. Langmuir **20,** 448, 2004.
35. Antipov, A.A., Sukhorukov, G.B., Donath, E., and Mohwald, H. Sustained release properties of polyelectrolyte multilayer capsules. J Phys Chem B **105,** 2281, 2001.
36. Caruso, F., and Mohwald, H. Protein multilayer formation on colloids through a stepwise self-assembly technique. J Am Chem Soc **121,** 6039, 1999.
37. Lvov, Y.M., Lu, Z.Q., Schenkman, J.B., Zu, X.L., and Rusling, J.F. Direct electrochemistry of myoglobin and cytochrome p450(cam) in alternate layer-by-layer films with DNA and other polyions. J Am Chem Soc **120,** 4073, 1998.
38. Berg, M.C., Yang, S.Y., Hammond, P.T., and Rubner, M.F. Controlling mammalian cell interactions on patterned polyelectrolyte multilayer surfaces. Langmuir **20,** 1362, 2004.
39. Rydzek, G., Thomann, J.S., Ben Ameur, N., Jierry, L., Mesini, P., Ponche, A., *et al.* Polymer multilayer films obtained by electrochemically catalyzed click chemistry. Langmuir **26,** 2816, 2010.
40. Lee, L., Cavalieri, F., Johnston, A.P., and Caruso, F. Influence of salt concentration on the assembly of DNA multilayer films. Langmuir **26,** 3415, 2010.
41. Kim, B.S., Park, S.W., and Hammond, P.T. Hydrogen-bonding layer-by-layer assembled biodegradable polymeric micelles as drug delivery vehicles from surfaces. ACS Nano **2,** 386, 2008.
42. Tang, Z., Wang, Y., Podsiadlo, P., and Kotov, N. Biomedical applications of layer-by-layer assembly: from biomimetics to tissue engineering. Adv Mater **18,** 3203, 2006.
43. Macdonald, M.L., Rodriguez, N.M., Shah, N.J., and Hammond, P.T. Characterization of tunable FGF-2 releasing polyelectrolyte multilayers. Biomacromolecules **11,** 2053, 2010.
44. Hajicharalambous, C.S., Lichter, J., Hix, W.T., Swierczewska, M., Rubner, M.F., and Rajagopalan, P. Nano- and sub-micron porous polyelectrolyte multilayer assemblies: biomimetic surfaces for human corneal epithelial cells. Biomaterials **30,** 4029, 2009.
45. Janorkar, A.V., Rajagopalan, P., Yarmush, M.L., and Megeed, Z. The use of elastin-like polypeptide-polyelectrolyte

complexes to control hepatocyte morphology and function *in vitro*. Biomaterials **29,** 625, 2008.
46. Kidambi, S., Lee, I., and Chan, C. Controlling primary hepatocyte adhesion and spreading on protein-free polyelectrolyte multilayer films. J Am Chem Soc **126,** 16286, 2004.
47. Kidambi, S., Udpa, N., Schroeder, S.A., Findlan, R., Lee, I., and Chan, C. Cell adhesion on polyelectrolyte multilayer coated polydimethylsiloxane surfaces with varying topographies. Tissue Eng **13,** 2105, 2007.
48. Kidambi, S., Sheng, L., Yarmush, M.L., Toner, M., Lee, I., and Chan, C. Patterned co-culture of primary hepatocytes and fibroblasts using polyelectrolyte multilayer templates. Macromol Biosci **7,** 344, 2007.
49. Thierry, B., Kujawa, P., Tkaczyk, C., Winnik, F.M., Bilodeau, L., and Tabrizian, M. Delivery platform for hydrophobic drugs: prodrug approach combined with self-assembled multilayers. J Am Chem Soc **127,** 1626, 2005.
50. Swiston, A.J., Cheng, C., Um, S.H., Irvine, D.J., Cohen, R.E., and Rubner, M.F. Surface functionalization of living cells with multilayer patches. Nano Lett **8,** 4446, 2008.
51. Daubine, F., Cortial, D., Ladam, G., Atmani, H., Haikel, Y., Voegel, J.C., *et al.* Nanostructured polyelectrolyte multilayer drug delivery systems for bone metastasis prevention. Biomaterials **30,** 6367, 2009.
52. Engler, A.J., Richert, L., Wong, J.Y., Picart, C., and Discher, D.E. Surface probe measurements of the elasticity of sectioned tissue, thin gels and polyelectrolyte multilayer films: correlations between substrate stiffness and cell adhesion. Surf Sci **570,** 142, 2004.
53. Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. Matrix elasticity directs stem cell lineage specification. Cell **126,** 677, 2006.
54. Tryoen-Toth, P., Vautier, D., Haikel, Y., Voegel, J.C., Schaaf, P., Chluba, J., *et al.* Viability, adhesion, and bone phenotype of osteoblast-like cells on polyelectrolyte multilayer films. J Biomed Mater Res **60,** 657, 2002.
55. Thompson, M.T., Berg, M.C., Tobias, I.S., Rubner, M.F., and Van Vliet, K.J. Tuning compliance of nanoscale polyelectrolyte multilayers to modulate cell adhesion. Biomaterials **26,** 6836, 2005.
56. Richert, L., Lavalle, P., Vautier, D., Senger, B., Stoltz, J.F., Schaaf, P., *et al.* Cell interactions with polyelectrolyte multilayer films. Biomacromolecules **3,** 1170, 2002.
57. Ren, K.F., Crouzier, T., Roy, C., and Picart, C. Polyelectrolyte multilayer films of controlled stiffness modulate myoblast cell differentiation. Adv Funct Mater **18,** 1378, 2008.
58. Benkirane-Jessel, N., Lavalle, P., Meyer, F., Audouin, F., Frisch, B., Schaaf, P., *et al.* Control of monocyte morphology on and response to model surfaces for implants equipped with anti-inflammatory agents. Adv Mater **16,** 1507, 2004.
59. Thierry, B., Winnik, F.M., Merhi, Y., and Tabrizian, M. Nanocoatings onto arteries via layer-by-layer deposition: toward the *in vivo* repair of damaged blood vessels. J Am Chem Soc **125,** 7494, 2003.
60. Schultz, P., Vautier, D., Richert, L., Jessel, N., Haikel, Y., Schaaf, P., *et al.* Polyelectrolyte multilayers functionalized by a synthetic analogue of an anti-inflammatory peptide, alpha-MSH, for coating a tracheal prosthesis. Biomaterials **26,** 2621, 2005.
61. Stevens, M.M., and George, J.H. Exploring and engineering the cell surface interface. Science **310,** 1135, 2005.
62. Discher, D.E., Janmey, P., and Wang, Y.L. Tissue cells feel and respond to the stiffness of their substrate. Science **310,** 1139, 2005.
63. Singhvi, R., Stephanopoulos, G., and Wang, D.I.C. Effects of substratum morphology on cell physiology—review. Biotechnol Bioeng **43,** 764, 1994.
64. Salloum, D.S., Olenych, S.G., Keller, T.C.S., and Schlenoff, J.B. Vascular smooth muscle cells on polyelectrolyte multilayers: hydrophobicity-directed adhesion and growth. Biomacromolecules **6,** 161, 2005.
65. Picart, C., Mutterer, J., Richert, L., Luo, Y., Prestwich, G.D., Schaaf, P., *et al.* Molecular basis for the explanation of the exponential growth of polyelectrolyte multilayers. Proc Natl Acad Sci U S A **99,** 12531, 2002.
66. Haynie, D.T., Zhang, L., Rudra, J.S., Zhao, W.H., Zhong, Y., and Palath, N. Polypeptide multilayer films. Biomacromolecules **6,** 2895, 2005.
67. Porcel, C., Lavalle, P., Decher, G., Senger, B., Voegel, J.C., and Schaaf, P. Influence of the polyelectrolyte molecular weight on exponentially growing multilayer films in the linear regime. Langmuir **23,** 1898, 2007.
68. Garza, J.M., Jessel, N., Ladam, G., Dupray, V., Muller, S., Stoltz, J.F., *et al.* Polyelectrolyte multilayers and degradable polymer layers as multicompartment films. Langmuir **21,** 12372, 2005.
69. Richert, L., Schneider, A., Vautier, D., Vodouhe, C., Jessel, N., Payan, E., *et al.* Imaging cell interactions with native and crosslinked polyelectrolyte multilayers. Cell Biochem Biophys **44,** 273, 2006.
70. Yang, S.Y., Lee, D., Cohen, R.E., and Rubner, M.F. Bioinert solution-cross-linked hydrogen-bonded multilayers on colloidal particles. Langmuir **20,** 5978, 2004.
71. Li, Q., Quinn, J.F., and Caruso, F. Nanoporous polymer thin films via polyelectrolyte templating. Adv Mater **17,** 2058, 2005.
72. Staros, J.V., Wright, R.W., and Swingle, D.M. Enhancement by N-hydroxysulfosuccinimide of water-soluble carbodiimide-mediated coupling reactions. Anal Biochem **156,** 220, 1986.
73. Mauser, T., Dejugnat, C., and Sukhorukov, G.B. Reversible pH-dependent properties of multilayer microcapsules made of weak polyelectrolytes. Macromol Rapid Commun **25,** 1781, 2004.
74. Koktysh, D.S., Liang, X.R., Yun, B.G., Pastoriza-Santos, I., Matts, R.L., Giersig, M., *et al.* Biomaterials by design: layer-by-layer assembled ion-selective and biocompatible films of TiO2 nanoshells for neurochemical monitoring. Adv Funct Mater **12,** 255, 2002.
75. Croll, T.I., O'Connor, A.J., Stevens, G.W., and Cooper-White, J.J. A blank slate? Layer-by-layer deposition of hyaluronic acid and chitosan onto various surfaces. Biomacromolecules **7,** 1610, 2006.
76. Kim, S.J., Lee, C.K., Lee, Y.M., Kim, I.Y., and Kim, S.I. Electrical/pH-sensitive swelling behavior of polyelectrolyte hydrogels prepared with hyaluronic acid-poly(vinyl alcohol) interpenetrating polymer networks. Reactive Funct Polym **55,** 291, 2003.
77. Lubig, R., Kusch, P., Roper, K., and Zahn, H. On the mechanism of protein crosslinking with glutaraldehyde. Monatsh Chem **112,** 1313, 1981.
78. Olek, M., Ostrander, J., Jurga, S., Mohwald, H., Kotov, N., Kempa, K., *et al.* Layer-by-layer assembled composites from multiwall carbon nanotubes with different morphologies. Nano Lett **4,** 1889, 2004.

79. Tong, W.J., Gao, C.Y., and Mohwald, H. Manipulating the properties of polyelectrolyte microcapsules by glutaraldehyde cross-linking. Chem Mater **17,** 4610, 2005.
80. Wittmer, C.R., Phelps, J.A., Saltzman, W.M., and Van Tassel, P.R. Fibronectin terminated multilayer films: protein adsorption and cell attachment studies. Biomaterials **28,** 851, 2007.
81. Brunot, C., Ponsonnet, L., Lagneau, C., Farge, P., Picart, C., and Grosgogeat, B. Cytotoxicity of polyethyleneimine (PEI), precursor base layer of polyelectrolyte multilayer films. Biomaterials **28,** 632, 2007.
82. Fischer, D., Li, Y., Ahlemeyer, B., Krieglstein, J., and Kissel, T. *In vitro* cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. Biomaterials **24,** 1121, 2003.
83. Richert, L., Boulmedais, F., Lavalle, P., Mutterer, J., Ferreux, E., Decher, G., *et al.* Improvement of stability and cell adhesion properties of polyelectrolyte multilayer films by chemical cross-linking. Biomacromolecules **5,** 284, 2004.
84. Abrams, G.A., Schaus, S.S., Goodman, S.L., Nealey, P.F., and Murphy, C.J. Nanoscale topography of the corneal epithelial basement membrane and Descemet's membrane of the human. Cornea **19,** 57, 2000.
85. Beuerman, R.W., and Pedroza, L. Ultrastructure of the human cornea. Microsc Res Tech **33,** 320, 1996.
86. Griffith, L.G., and Swartz, M.A. Capturing complex 3D tissue physiology *in vitro*. Nat Rev Mol Cell Biol **7,** 211, 2006.
87. Fery, A., Scholer, B., Cassagneau, T., and Caruso, F. Nanoporous thin films formed by salt-induced structural changes in multilayers of poly(acrylic acid) and poly (allylamine). Langmuir **17,** 3779, 2001.
88. Chien, H.W., Chang, T.Y., and Tsai, W.B. Spatial control of cellular adhesion using photo-crosslinked micropatterned polyelectrolyte multilayer films. Biomaterials **30,** 2209, 2009.
89. Zhai, L., Cebeci, F.C., Cohen, R.E., and Rubner, M.F. Stable superhydrophobic coatings from polyelectrolyte multilayers. Nano Lett **4,** 1349, 2004.
90. Bongaerts, J.H., Cooper-White, J.J., and Stokes, J.R. Low biofouling chitosan-hyaluronic acid multilayers with ultra-low friction coefficients. Biomacromolecules **10,** 1287, 2009.
91. Hsu, H.P., Zanella, J.M., Peckham, S.M., and Spector, M. Comparing ectopic bone growth induced by rhBMP-2 on an absorbable collagen sponge in rat and rabbit models. J Orthop Res **24,** 1660, 2006.
92. Richardson, T.P., Peters, M.C., Ennett, A.B., and Mooney, D.J. Polymeric system for dual growth factor delivery. Nat Biotechnol **19,** 1029, 2001.
93. Kim, B.S., Smith, R.C., Poon, Z., and Hammond, P.T. MAD (multiagent delivery) nanolayer: delivering multiple therapeutics from hierarchically assembled surface coatings. Langmuir **25,** 14086, 2009.
94. Guimond, S., Maccarana, M., Olwin, B.B., Lindahl, U., and Rapraeger, A.C. Activating and inhibitory heparin sequences for Fgf-2 (basic Fgf)—distinct requirements for Fgf-1, Fgf-2, and Fgf-4. J Biol Chem **268,** 23906, 1993.
95. Picart, C., Elkaim, R., Richert, L., Audoin, T., Arntz, Y., Cardoso, M.D., *et al.* Primary cell adhesion on RGD-functionalized and covalently crosslinked thin polyelectrolyte multilayer films. Adv Funct Mater **15,** 83, 2005.
96. Chua, P.H., Neoh, K.G., Kang, E.T., and Wang, W. Surface functionalization of titanium with hyaluronic acid/chitosan polyelectrolyte multilayers and RGD for promoting osteoblast functions and inhibiting bacterial adhesion. Biomaterials **29,** 1412, 2008.
97. Ruoslahti, E., and Pierschbacher, M.D. New perspectives in cell-adhesion—Rgd and integrins. Science **238,** 491, 1987.
98. Hersel, U., Dahmen, C., and Kessler, H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. Biomaterials **24,** 4385, 2003.
99. Tsai, W.B., Chen, R.P.Y., Wei, K.L., Chen, Y.R., Liao, T.Y., Liu, H.L., *et al.* Polyelectrolyte multilayer films functionalized with peptides for promoting osteoblast functions. Acta Biomater **5,** 3467, 2009.
100. Yoo, D., Shiratori, S.S., and Rubner, M.F. Controlling bilayer composition and surface wettability of sequentially adsorbed multilayers of weak polyelectrolytes. Macromolecules **31,** 4309, 1998.
101. Rezania, A., and Healy, K.E. The effect of peptide surface density on mineralization of a matrix deposited by osteogenic cells. J Biomed Mater Res **52,** 595, 2000.
102. Chen, A.A., Khetani, S.R., Lee, S., Bhatia, S.N., and Van Vliet, K.J. Modulation of hepatocyte phenotype *in vitro* via chemomechanical tuning of polyelectrolyte multilayers. Biomaterials **30,** 1113, 2009.
103. Wittmer, C.R., Phelps, J.A., Lepus, C.M., Saltzman, W.M., Harding, M.J., and Van Tassel, P.R. Multilayer nanofilms as substrates for hepatocellular applications. Biomaterials **29,** 4082, 2008.
104. Dunn, J.C., Tompkins, R.G., and Yarmush, M.L. Long-term *in vitro* function of adult hepatocytes in a collagen sandwich configuration. Biotechnol Prog **7,** 237, 1991.
105. Dunn, J.C., Yarmush, M.L., Koebe, H.G., and Tompkins, R.G. Hepatocyte function and extracellular matrix geometry: long-term culture in a sandwich configuration. FASEB J **3,** 174, 1989.
106. Kidambi, S., Yarmush, R.S., Novik, E., Chao, P., Yarmush, M.L., and Nahmias, Y. Oxygen-mediated enhancement of primary hepatocyte metabolism, functional polarization, gene expression, and drug clearance. Proc Natl Acad Sci U S A **106,** 15714, 2009.
107. Bhatia, S.N., Yarmush, M.L., and Toner, M. Controlling cell interactions by micropatterning in co-cultures: hepatocytes and 3T3 fibroblasts. J Biomed Mater Res **34,** 189, 1997.
108. Bhatia, S.N., Balis, U.J., Yarmush, M.L., and Toner, M. Effect of cell-cell interactions in preservation of cellular phenotype: cocultivation of hepatocytes and nonparenchymal cells. FASEB J **13,** 1883, 1999.
109. Khetani, S.R., and Bhatia, S.N. Microscale culture of human liver cells for drug development. Nat Biotechnol **26,** 120, 2008.
110. Pittenger, M.F., Mackay, A.M., Beck, S.C., Jaiswal, R.K., Douglas, R., Mosca, J.D., *et al.* Multilineage potential of adult human mesenchymal stem cells. Science **284,** 143, 1999.
111. Metallo, C.M., Mohr, J.C., Detzel, C.J., de Pablo, J.J., Van Wie, B.J., and Palecek, S.P. Engineering the stem cell microenvironment. Biotechnol Prog **23,** 18, 2007.
112. Motaln, H., Schichor, C., and Lah, T.T. Human mesenchymal stem cells and their use in cell-based therapies. Cancer Am Cancer Soc **116,** 2519, 2010.
113. Noghero, A., Bussolino, F., and Gualandris, A. Role of the microenvironment in the specification of endothelial progenitors derived from embryonic stem cells. Microvasc Res **79,** 178, 2010.
114. Salmon, N., Paternotte, E., Decot, V., Stoltz, J.F., Menu, P., and Labrude, P. Polyelectrolyte multilayer films promote human cord blood stem cells differentiation into mature

endothelial cells exhibiting a stable phenotype. Biomed Mater Eng **19,** 349, 2009.
115. Berthelemy, N., Kerdjoudj, H., Gaucher, C., Schaaf, P., Stolz, J.F., Lacolley, P., *et al.* Polyelectrolyte films boost progenitor cell differentiation into endothelium-like monolayers. Adv Mater **20,** 2674, 2008.
116. Thebaud, N.B., Bareille, R., Daculsi, R., Bourget, C., Remy, M., Kerdjoudj, H., *et al.* Polyelectrolyte multilayer films allow seeded human progenitor-derived endothelial cells to remain functional under shear stress *in vitro*. Acta Biomater **6,** 1437, 2010.
117. Kaushal, S., Amiel, G.E., Guleserian, K.J., Shapira, O.M., Perry, T., Sutherland, F.W., *et al.* Functional small-diameter neovessels created using endothelial progenitor cells expanded *ex vivo*. Nat Med **7,** 1035, 2001.
118. Jan, E., and Kotov, N.A. Successful differentiation of mouse neural stem cells on layer-by-layer assembled single-walled carbon nanotube composite. Nano Lett **7,** 1123, 2007.
119. Liu, Z.M., Gu, Q., Xu, Z.K., and Groth, T. Synergistic effect of polyelectrolyte multilayers and osteogenic growth medium on differentiation of human mesenchymal stem cells. Macromol Biosci **10,** 1043, 2010.
120. Tsai, H.A., Wu, R.R., Lee, I.C., Chang, H.Y., Shen, C.N., and Chang, Y.C. Selection, enrichment, and maintenance of self-renewal liver stem/progenitor cells utilizing polypeptide polyelectrolyte multilayer films. Biomacromolecules **11,** 994, 2010.
121. Okumoto, K., Saito, T., Hattori, E., Ito, J., Adachi, T., Takeda, T., *et al.* Differentiation of bone marrow cells into cells that express liver-specific genes *in vitro*: implication of the Notch signals in differentiation. Biochem Biophys Res Commun **304,** 691, 2003.
122. Tsuchiya, A., Heike, T., Fujino, H., Shiota, M., Umeda, K., Yoshimoto, M., *et al.* Long-term extensive expansion of mouse hepatic stem/progenitor cells in a novel serum-free culture system. Gastroenterology **128,** 2089, 2005.
123. Liao, T., Moussallem, M.D., Kim, J., Schlenoff, J.B., and Ma, T. N-isopropylacrylamide-based thermoresponsive polyelectrolyte multilayer films for human mesenchymal stem cell expansion. Biotechnol Prog **26,** 1705, 2010.
124. Wood, K.C., Chuang, H.F., Batten, R.D., Lynn, D.M., and Hammond, P.T. Controlling interlayer diffusion to achieve sustained, multiagent delivery from layer-by-layer thin films. Proc Natl Acad Sci U S A **103,** 10207, 2006.
125. Caruso, F., Yang, W.J., Trau, D., and Renneberg, R. Microencapsulation of uncharged low molecular weight organic materials by polyelectrolyte multilayer self-assembly. Langmuir **16,** 8932, 2000.
126. Pang, Z., Feng, L., Hua, R., Chen, J., Gao, H., Pan, S., *et al.* Lactoferrin-conjugated biodegradable polymersome holding doxorubicin and tetrandrine for chemotherapy of Glioma rats. Mol Pharm **7,** 1995, 2010.
127. Liu, G., Ma, S., Li, S., Cheng, R., Meng, F., Liu, H., *et al.* The highly efficient delivery of exogenous proteins into cells mediated by biodegradable chimaeric polymersomes. Biomaterials **31,** 7575, 2010.
128. Kim, Y., Tewari, M., Pajerowski, J.D., Cai, S., Sen, S., Williams, J.H., *et al.* Polymersome delivery of siRNA and antisense oligonucleotides. J Control Release **134,** 132, 2009.
129. Discher, D.E., and Eisenberg, A. Polymer vesicles. Science **297,** 967, 2002.
130. Chandrawati, R., Hosta-Rigau, L., Vanderstraaten, D., Lokuliyana, S.A., Stadler, B., Albericio, F., *et al.* Engineering advanced capsosomes: maximizing the number of subcompartments, cargo retention, and temperature-triggered reaction. ACS Nano **4,** 1351, 2010.
131. Hosta-Rigau, L., Stadler, B., Yan, Y., Nice, E.C., Heath, J.K., Albericio, F., *et al.* Capsosomes with multilayered subcompartments: assembly and loading with hydrophobic cargo. Adv Funct Mater **20,** 59, 2010.
132. Glinel, K., Prevot, M., Krustev, R., Sukhorukov, G.B., Jonas, A.M., and Mohwald, H. Control of the water permeability of polyelectrolyte multilayers by deposition of charged paraffin particles. Langmuir **20,** 4898, 2004.
133. Benkirane-Jessel, N., Lavalle, P., Hubsch, E., Holl, V., Senger, B., Haikel, Y., *et al.* Short-time timing of the biological activity of functionalized polyelectrolyte multilayers. Adv Funct Mater **15,** 648, 2005.
134. Jessel, N., Atalar, F., Lavalle, P., Mutterer, J., Decher, G., Schaaf, P., *et al.* Bioactive coatings based on a polyelectrolyte multilayer architecture functionalized by embedded proteins. Adv Mater **15,** 692, 2003.
135. Diaspro, A., Silvano, D., Krol, S., Cavalleri, O., and Gliozzi, A. Single living cell encapsulation in nano-organized polyelectrolyte shells. Langmuir **18,** 5047, 2002.
136. Krol, S., Nolte, M., Diaspro, A., Mazza, D., Magrassi, R., Gliozzi, A., *et al.* Encapsulated living cells on microstructured surfaces. Langmuir **21,** 705, 2004.
137. Germain, M., Balaguer, P., Nicolas, J.C., Lopez, F., Esteve, J.P., Sukhorukov, G.B., *et al.* Protection of mammalian cell used in biosensors by coating with a polyelectrolyte shell. Biosens Bioelectron **21,** 1566, 2006.
138. Hillberg, A.L., and Tabrizian, M. Biorecognition through layer-by-layer polyelectrolyte assembly: *in-situ* hybridization on living cells. Biomacromolecules **7,** 2742, 2006.
139. Veerabadran, N.G., Goli, P.L., Stewart-Clark, S.S., Lvov, Y.M., and Mills, D.K. Nanoencapsulation of stem cells within polyelectrolyte multilayer shells. Macromol Biosci **7,** 877, 2007.
140. Lutkenhaus, J.L., Hrabak, K.D., McEnnis, K., and Hammond, P.T. Elastomeric flexible free-standing hydrogen-bonded nanoscale assemblies. J Am Chem Soc **127,** 17228, 2005.
141. Jaber, J.A., and Schlenoff, J.B. Mechanical properties of reversibly cross-linked ultrathin polyelectrolyte complexes. J Am Chem Soc **128,** 2940, 2006.
142. Richert, L., Arntz, Y., Schaaf, P., Voegel, J.C., and Picart, C. pH dependent growth of poly(L-lysine)/poly(L-glutamic) acid multilayer films and their cell adhesion properties. Surf Sci **570,** 13, 2004.
143. Lulevich, V.V., and Vinogradova, O.I. Effect of pH and salt on the stiffness of polyelectrolyte multilayer microcapsules. Langmuir **20,** 2874, 2004.
144. Nolte, A.J., Cohen, R.E., and Rubner, M.F. A two-plate buckling technique for thin film modulus measurements: applications to polyelectrolyte multilayers. Macromolecules **39,** 4841, 2006.
145. Zheng, H., Berg, M.C., Rubner, M.F., and Hammond, P.T. Controlling cell attachment selectively onto biological polymer-colloid templates using polymer-on-polymer stamping. Langmuir **20,** 7215, 2004.
146. Berg, M.C., Choi, J., Hammond, P.T., and Rubner, M.F. Tailored micropatterns through weal: polyelectrolyte stamping. Langmuir **19,** 2231, 2003.
147. Vautier, D., Karsten, V., Egles, C., Chluba, J., Schaaf, P., Voegel, J.C., *et al.* Polyelectrolyte multilayer films modulate cytoskeletal organization in chondrosarcoma cells. J Biomater Sci Polym Ed **13,** 713, 2002.

148. Hwang, J.J., Jelacic, S., Samuel, N.T., Maier, R.V., Campbell, C.T., Castner, D.G., *et al.* Monocyte activation on polyelectrolyte multilayers. J Biomater Sci Polym Ed **16,** 237, 2005.
149. Schneider, A., Francius, G., Obeid, R., Schwinte, P., Hemmerle, J., Frisch, B., *et al.* Polyelectrolyte multilayers with a tunable Young's modulus: influence of film stiffness on cell adhesion. Langmuir **22,** 1193, 2006.
150. Schneider, A., Bolcato-Bellemin, A.L., Francius, G., Jedrzejwska, J., Schaaf, P., Voegel, J.C., *et al.* Glycated polyelectrolyte multilayer films: differential adhesion of primary versus tumor cells. Biomacromolecules **7,** 2882, 2006.
151. Schneider, A., Richert, L., Francius, G., Voegel, J.C., and Picart, C. Elasticity, biodegradability and cell adhesive properties of chitosan/hyaluronan multilayer films. Biomed Mater **2,** S45, 2007.
152. Schneider, A., Picart, C., Senger, B., Schaaf, P., Voegel, J.C., and Frisch, B. Layer-by-layer films from hyaluronan and amine-modified hyaluronan. Langmuir **23,** 2655, 2007.
153. Brunot, C., Grosgogeat, B., Picart, C., Lagneau, C., Jaffrezic-Renault, N., and Ponsonnet, L. Response of fibroblast activity and polyelectrolyte multilayer films coating titanium. Dent Mater **24,** 1025, 2008.
154. Boura, C., Kerdjoudj, H., Moby, V., Vautier, D., Dumas, D., Schaaf, P., *et al.* Initial adhesion of endothelial cells on polyelectrolyte multilayer films. Biomed Mater Eng **16,** S115, 2006.
155. Kerdjoudj, H., Boura, C., Moby, V., Montagne, K., Schaaf, P., Voegel, J.C., *et al.* Re-endothelialization of human umbilical arteries treated with polyelectrolyte multilayers: a tool for damaged vessel replacement. Adv Funct Mater **17,** 2667, 2007.
156. Moby, V., Boura, C., Kerdjoudj, H., Voegel, J.C., Marchal, L., Dumas, D., *et al.* Poly(styrenesulfonate)/poly (allylamine) multilayers: a route to favor endothelial cell growth on expanded poly(tetrafluoroethylene) vascular grafts. Biomacromolecules **8,** 2156, 2007.
157. Seo, J., Lee, H., Jeon, J., Jang, Y., Kim, R., Char, K., *et al.* Tunable layer-by-layer polyelectrolyte platforms for comparative cell assays. Biomacromolecules **10,** 2254, 2009.
158. Zhang, X., Kovtun, A., Mendoza-Palomares, C., Oulad-Abdelghani, M., Fioretti, F., Rinckenbach, S., *et al.* siRNA-loaded multi-shell nanoparticles incorporated into a multilayered film as a reservoir for gene silencing. Biomaterials **31,** 6013, 2010.
159. Zhang, X., Sharma, K.K., Boeglin, M., Ogier, J., Mainard, D., Voegel, J.C., *et al.* Transfection ability and intracellular DNA pathway of nanostructured gene-delivery systems. Nano Lett **8,** 2432, 2008.
160. Lin, Q.K., Ren, K.F., and Ji, J. Hyaluronic acid and chitosan-DNA complex multilayered thin film as surface-mediated nonviral gene delivery system. Colloids Surf B Biointerfaces **74,** 298, 2009.
161. Jessel, N., Oulad-Abdelghani, M., Meyer, F., Lavalle, P., Haikel, Y., Schaaf, P., *et al.* Multiple and time-scheduled *in situ* DNA delivery mediated by beta-cyclodextrin embedded in a polyelectrolyte multilayer. Proc Natl Acad Sci U S A **103,** 8618, 2006.
162. Chluba, J., Voegel, J.C., Decher, G., Erbacher, P., Schaaf, P., and Ogier, J. Peptide hormone covalently bound to polyelectrolytes and embedded into multilayer architectures conserving full biological activity. Biomacromolecules **2,** 800, 2001.
163. Gangloff, S.C., Ladam, G., Dupray, V., Fukase, K., Brandenburg, K., Guenounou, M., *et al.* Biologically active lipid A antagonist embedded in a multilayered polyelectrolyte architecture. Biomaterials **27,** 1771, 2006.
164. Leguen, E., Chassepot, A., Decher, G., Schaaf, P., Voegel, J.C., and Jessel, N. Bioactive coatings based on polyelectrolyte multilayer architectures functionalized by embedded proteins, peptides or drugs. Biomol Eng **24,** 33, 2007.
165. Vodouhe, C., Le Guen, E., Garza, J.M., Francius, G., Dejugnat, C., Ogier, J., *et al.* Control of drug accessibility on functional polyelectrolyte multilayer films. Biomaterials **27,** 4149, 2006.
166. Nadiri, A., Kuchler-Bopp, S., Mjahed, H., Hu, B., Haikel, Y., Schaaf, P., *et al.* Cell apoptosis control using BMP4 and noggin embedded in a polyelectrolyte multilayer film. Small **3,** 1577, 2007.
167. Shukla, A., Fleming, K.E., Chuang, H.F., Chau, T.M., Loose, C.R., Stephanopoulos, G.N., *et al.* Controlling the release of peptide antimicrobial agents from surfaces. Biomaterials **31,** 2348, 2010.

Address correspondence to:
Padmavathy Rajagopalan, Ph.D.
Department of Chemical Engineering
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061

E-mail: padmar@vt.edu

Received: September 16, 2010
Accepted: January 3, 2011
Online Publication Date: February 10, 2011