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INVITED REVIEW PAPER

Functionalization of polymer multilayer thin films for novel biomedical applications

Yeongseon Jang, Saibom Park, and Kookheon Char†

Intelligent Hybrids Research Center, School of Chemical and Biological Engineering,
The WCU Program of Chemical Convergence for Energy & Environment,
Seoul National University, Seoul 151-744, Korea
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Abstract−The design of functional polymer multilayer thin films with nanometer scale control is of great interest for biomedical applications such as tissue engineering, targeted drug delivery, controlled release system, and regenerative medicine. Various functions and properties of polymer thin films can be easily programmed and realized by the layer-by-layer assembly strategy, which is a facile and versatile deposition method to prepare well-defined biomedical multilayer platforms due to its benign process to prepare films under mild conditions and the capability of incorporating bioactive materials at a desired location within the films. Particularly, the fine tuning of physicochemical and biological properties of multilayer thin films is significantly important for designing novel biomedical platforms capable of adjusting the cellular functions. In this review, we focus on the overall background of the layer-by-layer assembly as well as the tuning of multilayer film properties and the programming of biological functions into the polymer thin films with a view on the control of cellular functions. Furthermore, we highlighted the recent achievements toward the design of novel biomedical platforms based on functionalized polymer multilayer thin films.

Key words: Layer-by-layer Assembly, Multilayer Thin Film(s), Bio-functionalization, Cellular Function(s), Biomedical Platform(s)

INTRODUCTION

The preparation and tuning of functional polymer thin films at the molecular level have caught the significant attention of many researchers in the field of nanobio-medicine due to numerous potential applications. Ranging from biocompatible implant coatings to tissue engineering scaffolds, considerable efforts have been devoted to design polymeric thin films with new biological functionalities. In other words, the design of thin films with precise control of their structures and properties for targeted biomedical applications has become important and critical issues as researchers have started to appreciate the influence of surface or film properties on cellular functions such as adhesion, proliferation, motility, differentiation and so forth.

Among many fabrication techniques available to realize functional polymer thin films, the layer-by-layer (LbL) assembly has been considered to be the one of the most efficient and practical methods owing to its simplicity in fabrication steps as well as versatility in the choice of both depositing materials and substrates. Moreover, the LbL deposition technique is ideally suited to biomedical applications because the LbL assembly could be typically performed in aqueous solution. Consequently, the LbL deposition has been utilized to modify or functionalize the multilayer films for biomedical purpose by the precise control of thin film properties (i.e., modulus, charge density, release characteristics) as well as the incorporation of drugs or active ingredients at a desired position within the multilayer films.

†To whom correspondence should be addressed.
E-mail: khchar@plaza.snu.ac.kr

In this review, we will focus on functional polymer multilayer thin films taking full advantage of the potential offered by the LbL assembly, particularly geared toward novel biomedical applications. This review is intended to give the overall background on the LbL assembly from the basic building-up principle to tuning and functionalization of thin film properties as well as to provide several applications of the functional multilayer films in the biomedical area. We hope that this review, based on the engineering and bio-functionalization of polymeric multilayer thin films, could ultimately contribute to open up new possibilities to design flexible and multifunctional polymer platforms for many biomedical applications.

THE LAYER-BY-LAYER (LBL) ASSEMBLY

The layer-by-layer (LbL) assembly technique is one of the simplest and most versatile methods for preparing multifunctional polymer thin films, taking advantage of various intermolecular interactions among paired species. First introduced by Decher and coworkers in the early 1990s [1,2], diverse applications such as chemical and biological sensors, drug delivery systems, photovoltaics, and electrochromic devices have been demonstrated based on the LbL assembly. The general introduction of the LbL assembly as well as various applications of the LbL-assembled multilayer thin films has already been well described in many reviews [3-10]. Consequently, in this section, we would like to focus on three important aspects of the LbL assembly in order to better understand LbL platforms for biomedical applications: 1) the build-up principles employing different driving forces and deposition methods currently available for multilayer formation; 2) the process variables to tune thin film properties; and 3) the release behavior of LbL multilayred films

triggered by external stimuli.

1. Basic Build-up Principles of Layer-by-layer (LbL) Assembly

The concept of building up multilayer films was first suggested by Iler, who reported the preparation of multilayer films by combining positively and negatively charged colloidal particles [11]. Some decades later, Decher and coworkers realized multilayer films prepared from oppositely charged polyelectrolytes [1,2]. Afterward, the number of publications dealing with the LbL multilayer films expanded dramatically, ranging from the basic principles addressing LbL deposition mechanism to numerous potential applications for the last two decades, as shown in Fig. 1.

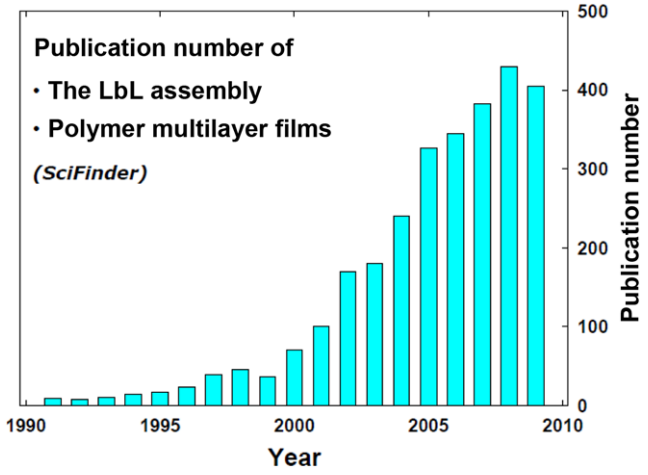

Fig. 1. The chronological trend in publication number under the title of 'the LbL assembly' and 'polymer multilayer films' of SCI journals searched by SCI Finder.

Conventional LbL assembly is based on the dipping method (i.e., dip-assisted LbL assembly). In the case of the dip-assisted LbL assembly, a substrate is alternatively immersed into two different aqueous solutions containing pairing polymers, with rinsing steps in between. In this case, adsorbed species or polymeric chains diffuse to and adsorb on the substrate from aqueous solution mediated by various intermolecular interactions, followed by the slow molecular rearrangement on the surface, as depicted in Fig. 2. The conventional LbL assembly based on the solution dipping has recently been extended to spin-assisted [12], spray-assisted [13], and microfluidic-assisted LbL assembly [14]. The alternative LbL deposition methods further expand control parameters of the conventional LbL assembly. In addition, detailed LbL deposition methods are also known to play an important role in terms of controlling final film characteristics. For example, the spin-assisted LbL deposition method has been of particular interest as opposed to the conventional dip-assisted LbL deposition because the spin method offers several merits such as drastically reduced process time for the preparation of well-defined multilayer films with highly ordered internal structure as well as minimal solvent usage and uniform surface morphology and properties.

The main driving forces to prepare multilayer thin films based on the LbL assembly are various molecular interactions. Those interactions are not simply limited to the electrostatic interactions but other intermolecular interactions such as hydrogen bonding, covalent bonding, donor-acceptor interaction (charge-transfer interaction), π-π interactions, and hydrophobic interactions have also been tested to prepare the multilayer films. These diverse intermolecular interactions also enable us to utilize functional nano-objects such as polymeric micelles, nanoparticles, and biomolecules (such as DNA, pro-

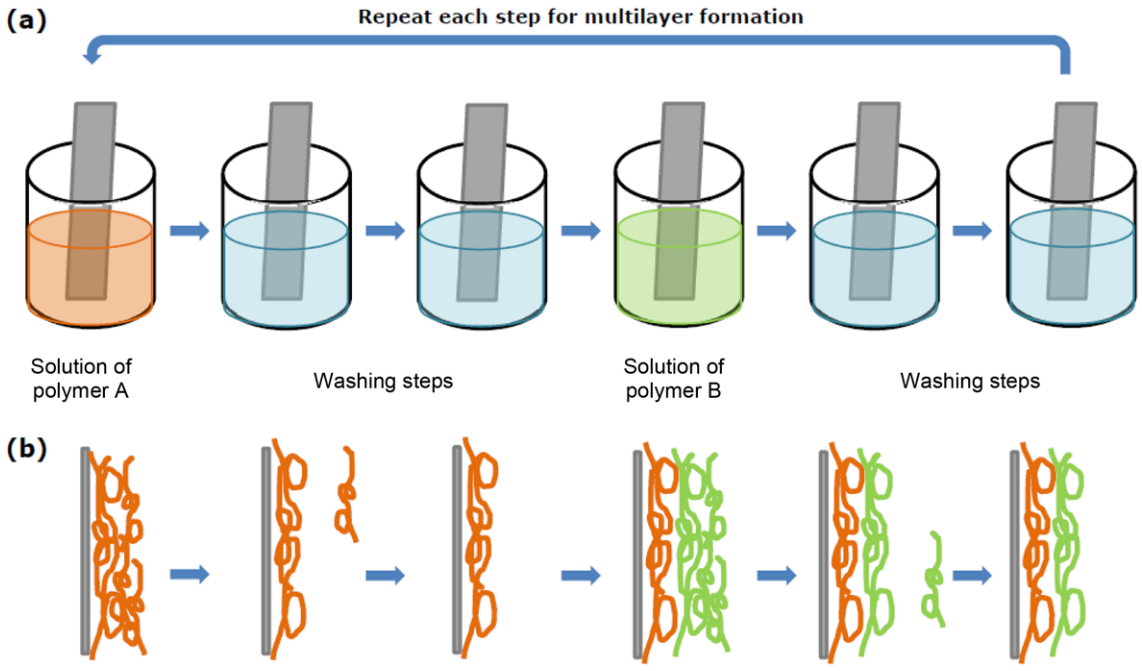

Fig. 2. Schematics on (a) the film deposition process by the dip-assisted layer-by-layer (LbL) deposition and (b) the change in polymer chain conformation during the LbL deposition showing the adsorption/desorption steps through the diffusion and rearrangement of polymer chains on the surface of a substrate.

teins, polysaccharides to name a few) to prepare functional LbL multilayer films.

Apart from intermolecular interactions for LbL deposition, intrinsic properties of adsorbing species such as chemical structures, chain length and stiffness and physical and chemical characteristics of suspending medium such as pH and the amount and type of salts used have also been identified as important process parameters to control the multilayer formation [15]. That is, important features of LbL films such as thickness increment could be finely tuned by the adsorption conditions such as ionic strength, temperature, solution pH, solvent polarity and so on in addition to the range of interactions for a selected intermolecular interaction.

Furthermore, the correlation between internal structure and functional properties of LbL multilayer films has keenly been considered in order to design stimuli-responsive surfaces and/or novel polymeric platforms for biomedical applications. Interested readers can refer to a review by von Klitzing in 2006 [16] for the role of internal structure of LbL films in both the dynamics of multilayer films and their functional properties.

2. Fine Tuning of Thin Multilayer Film Properties

The precise control and characterization of various properties of multilayer films such as mechanical stiffness is of great importance, particularly for biomedical applications. It has been well documented that mechanical properties of polymeric multilayer films play an important role in controlling the interactions between a surface and cells [17] because cells prefer to adhere on hard surfaces, in general [18]. Here, diverse methods to finely tune the properties of multilayer thin films will be reviewed with a particular emphasis on the effect of thin film properties for biological functions.

The assembly or post-assembly *pH condition* is the first parameter to control the film growth, charge density, and swelling or release behavior of multilayer films involving weak polyelectrolytes. Since the dissociation of carboxylic acidic and amine functional groups in weak polyelectrolyte chains (such as poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH)) is dependent on pH, the multilayer films prepared from such weak polyelectrolytes are quite sensitive to the solution pH. Most notably, Rubner and coworkers have published several papers dealing with pH-dependent adsorption behavior [19] and pH-triggered swelling transition in weak polyelectrolyte multilayer films [20]. They investigated the effect of assembly pH conditions employed to prepare (PAH/PAA) multilayer films on the physicochemical properties such as mechanical stiffness, surface roughness, film thickness, and wettability. In addition, the pH control also allows the ionic state of the multilayer films to be finely tuned, which, in turn, determines the degree of hydration and swelling of the films. By the fine tuning of the swelling states of (PAH/PAA) multilayer films deposited at different pH combinations, it has been demonstrated that the adhesion of fibroblast cells could be controlled [21].

Another important process parameter to tune the film properties is *ionic strength*. Ionic strength, typically determined by the type and amount of salts, is a powerful parameter to control permeability and rigidity of multilayered films [15]. In contrast to pH, the ionic strength is involved in multilayred films consisting of strong polyelectrolytes because the ionic strength has a net effect of screening their electrostatic charges. Schelenoff and his coworkers are leading in this endeavor, focusing on the relationship of ionic strength controlled by salts and internal structure of multilayer films involving poly(styrene sulfonate) (PSS) and poly(diallyl dimethyl ammonium) (PDDA) and reported that the films are more swollen, smoothed, and softened as a large amount of salts was added [22,23]. In addition, Fery et al. demonstrated salt-induced nanoporous LbL thin films [24], with potential applications in stimuli-responsive drug delivery systems.

Thermal or chemical *crosslinking* has also become one of common treatments to adjust the mechanical properties of polymeric

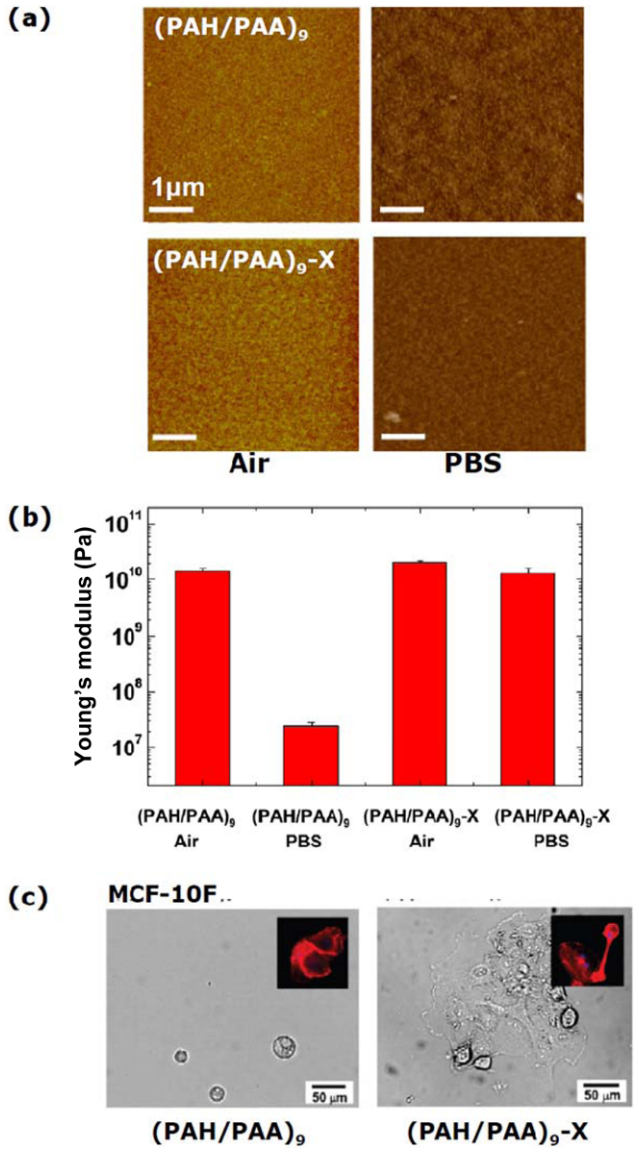

Fig. 3. (a) Surface images of $(PAH/PAA)_9$ and crosslinked $(PAH/PAA)_9$-X LbL films in air (left column) and in PBS buffer solution (right column) measured by liquid atomic force microscopy. (b) Young's moduli of $(PAH/PAA)_9$ and $(PAH/PAA)_9$-X films in both air and PBS buffer solution measured by the nanoindentation of liquid atomic force microscopy. (c) Optical images of noncancerous fibrocystic MCF 10F cells after 7 days of culture on $(PAH/PAA)_9$ and $(PAH/PAA)_9$/PAH-X. Inset images showing actin and nuclear-stained cells. This figure is reproduced from [17] with permission. Copyright 2009 American Chemical Society.

multilayer films. For example, the thermal treatment at high temperature increases the rigidity of polymer multilayer films by inducing the formation of amide or imide covalent bonds within the films. Char et al. have shown that the LbL film's rigidity was increased by the heat-assisted crosslinking reaction between amine groups of PAH and carboxylic groups of PAA and the crosslinked soft multilayer substrate showed a distinct spreading behavior of cancer cells as well as the significant change in cell phenotype, as shown in Fig. 3. Consequently, the polyelectrolyte multilayered film platforms with finely tuned mechanical properties have potential for the development of comparative cell assays [17]. Moreover, the carbodiimide chemistry can also be utilized to realize covalent amide bonds by crosslinking the carboxylic groups with the amine groups in mild conditions. One of representative crosslinkers is 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC). Picart et al. have demonstrated the crosslinking in chitosan and hyaluronan-based multilayer films (CHI/HA) by using a water soluble EDC and that the crosslinked films were more resistant than the pristine counterparts against the enzymatic degradation [25].

3. Stimuli-triggered Release from Functional Polymer Multilayer Films

In addition to the build-up of multilayered thin films with well-ordered structure, precise control on the degradation or release of polymer multilayer thin films has also been actively pursued during the last few years, leading to important advances in controlled release and drug delivery systems. Particular emphasis had been placed on the release behavior of such polymer-based multilayer films triggered by external stimuli for a variety of biomedical applications. Among many experimental variables which could trigger the degradation of multilayer films, most frequently used triggering cues are pH, temperature, light, or enzymes for the controlled release of active ingredients from the multilayer films.

As previously mentioned, the multilayer films based on weak polyelectrolyte pairs are known to be quite sensitive to external solution pH. It is thus possible to control the incorporation or release of target molecules within the polymer multilayer films by varying the degree of film swelling as well as modulating the attractive or repulsive interactions between target molecules and acidic or basic functional groups contained in the polyelectrolyte chains as a function of solution pH. Hence, the triggering with solution pH has been widely explored to cause morphological changes in films or to control the permeability of polyelectrolyte multilayer capsules. Such pH-triggered changes in the morphology of thin films can be used to release low molecular weight compounds such as drugs [26] or growth factors [27]. Intriguing results for the regulation of release behavior by external solution pH have already been well documented in several reviews [5,28].

Using thermo-responsive polymers such as poly(N-isopropylacrylamide) (PNIPAm), LbL-based multilayered films have also shown the temperature-driven tuning of uptake and release behavior of active ingredients within the films. PNIPAm is known to show the reversible volume transition at the lower critical solution temperature (LCST) around 31 ºC, and the hydrophobical collapse and the excluded volume expansion of PNIPAm chains in aqueous solution can be easily tuned by external temperature. Caruso and coworkers have shown that a dye, Rhodamine B, could be reversibly loaded or released within or from the multilayer films containing PNIPAm by simply tuning the solution temperature and the release kinetics is utterly sensitive to temperature [29].

Furthermore, disintegration and erosion of polymeric multilayer films can also be triggered by light. Möhwald et al. developed multilayer thin films based on biopolymer pairs hyaluronic acid (HA) and poly-L-lysine (PLL), decorated with DNA and Au nanoparticles, to demonstrate the light-triggered drug delivery. They showed that such biofilms have a high loading capacity of Au nanoparticles and could be activated to release of DNA upon irradiation of near IR light due to Au nanoparticles incorporated within the films. In addition, the near IR activated release of drugs (e.g., dextran), loaded in the core region of microcapsules, has also been demonstrated with Au nanoparticles residing at the shell phase, opening up the release pathway by immobilized light-sensitive hot spots incorporated into functional polymer multilayer films [30].

Enzymatic degradation of multilayer films containing polysaccharides [25] or DNA [31] can also be utilized for controlled release systems. In recent years, many researches have also focused on the hydrolytically induced release triggered in physiological conditions for applications in therapeutic perspectives [32,33].

BIO-FUNCTIONALIZATION OF POLYMERIC MULTILAYER FILMS

We have so far reviewed the LbL assembly to prepare functional multilayer films and the methods to modulate film property itself as well as to control the degradation of multilayer films applied to the controlled release of biomedical systems. Such tunability of film properties by the LbL deposition has prompted numerous cases of biomedical applications ranging from smart surfaces adjusting cellular adhesion to drug delivery microcapsules. However, multilayer films based on synthetic polymers have some limitations for further study in biological functions of such multilayered films as an extracellular matrix (ECM). Consequently, leading research groups have tested a variety of different approaches to functionalize the polymeric multilayer films in biological perspectives and three different strategies to bio-functionalize the multilayer films will be focused on in this section.

1. Adsorption of Proteins or Specific Ligands onto Polymer Thin Films

The simplest method to add bio-functionality in multilayer films is the adsorption of proteins or specific ligands onto polymer multilayer films through the LbL assembly. Several studies have demonstrated modulating cellular functions such as differentiation and proliferation of cells on the polymer multilayer films simply by coating the final top layer with biomolecules known as specific cell receptors, for examples, fibronectins (FN) [34,35], arginine-glycine-aspartate aminoacid sequences (RGD) [36], laminins or collagens [37].

Tassel and coworkers, who first adsorbed proteins on LbL multilayer films, investigated the adsorption of FN on (PAH/PSS) multilayer films using optical waveguide lightmode spectroscopy (OWLS) and AFM. They found that the FN was adsorbed on positively charged PAH top-layered surfaces with fast initial rate and large adsorbed amount due to the negatively charged characteristics of FN [34]. Since FN is one of typical extracellular matrix (ECM) proteins to promote cell adhesion, Fn-coated polymer multilayer films can also be used to enhance the interaction between multilayer films and

living cells, thus representing a promising strategy toward *in vivo* applications such as tissue engineering [35]. Similarly, RGD peptides also promote the cell adhesion to artificial surfaces. Picart et al. functionalized (PLL/PGA) multilayer films by grafting RGD sequences to PGA (poly(L-glutamic acid)) backbones, investigating the effect of such RGD-functionalized multilayer films on the short-term adhesion as well as the long-term proliferation of primary osteoblast cells [36]. The RGD-grafted LbL films showed higher short-term adhesion and proliferation when compared with unfunctionalized pristine films. These results clearly elucidate that not only the physical properties such as mechanical stiffness but also the chemical properties adjusted by the coating of biomolecules such as RGD sequences play an important role for the control of cellular functions.

2. Construction of Multilayer Films Based on Natural Biomaterials

Various cell properties such as cellular adhesion, motility, and cytoskeleton organization are typically modulated by *in vivo* ECMs. Therefore, efforts to mimic the functions of original ECMs in animal cells have been made by the LbL assembly. One of the simplest methods is to use natural ECM components as building blocks for the LbL-assembled multilayer films. Since *in vivo* ECM proteins such as collagens [38], gelatins [39], glycoaminoglycans such as hyaluronans (HA) [40], chondroitin sulfates [41], and heparins [42] have high biocompatibility as well as possible self-degradability, the multilayer films prepared from such ECM building blocks can be easily applied to tissue engineering and biodegradable drug delivery systems without causing toxicity [15].

Lavalle et al. [43] described the buildup of ordered molecular assemblies based on the LbL deposition using a natural polysaccharide HA and collagen. They mainly focused on the deposition process as well as on the film characterization from the physicochemical point of view. They also presented several results related to the interaction of chondrosarcoma cells with these types of films.

Furthermore, several attempts to chemically modify non-bioactive surfaces were made by the LbL assembly with natural biomaterials to improve biocompatibility as well as to allow additional surface functionalization such as enzyme and protein immobilization through the robust covalent bonding [44,45]. Gao and coworkers [41] reported the surface modification of poly(ethylene terephthalate) (PET) films, due to the lack of bioactive functionality, by the LbL assembly based on the electrostatic adsorption of oppositely charged natural biopolymers, chitosan and chondroitin sulfate. When the modified PET films were adopted in treating the pathology of arteries and vessels, the surface modification of synthetic substrates with the ECM building blocks accelerated the endothelialization [46] of vascular grafts through the favorable interactions between synthetic materials and organisms tuned mainly at the materials' interface.

Cooper-White et al. [47] also investigated the LbL electrostatic deposition with very high molecular weight HAs and chitosans on poly(lactic-co-glycolic acid) (PLGA) films in order to prevent significant foreign body response and uncontrolled fibrotic encapsulation. A few years later, the same researchers also generated non-fouling surfaces based on the pair of HA and chitosan containing proteins or peptides with good temporal stability as well as the ability to direct biological outcomes for stem cell culture [48].

3. Surface Topography Realized on Polymer Multilayer Thin Films

Surface topography is known to be one of important parameters to influence the adhesion and spreading behavior of cells. As a result, many cell lines have been tested to assess the effect of topologically structured surfaces, which are modified with inorganic nanoparticles, nanotubes, or patterning, due to recent advances in micro- or nano-fabrication techniques allowing us to investigate the cells' behavior in nanostructured environment of interest [49,50]. More-

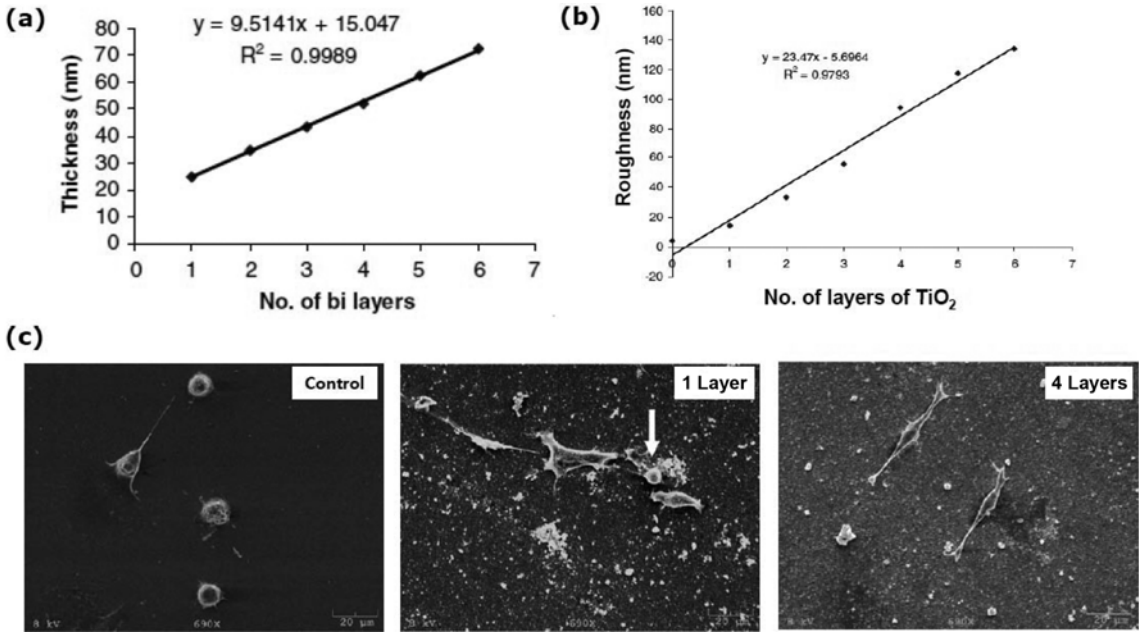

Fig. 4. The linear increase in (a) thickness and (b) surface roughness of (TiO_2/PSS) multilayer films prepared by the layer-by-layer deposition. (c) The SEM images of mouse mesenchymal stem cells on substrates: glass (left), one layer (middle) and four layers (right) of TiO_2 after 12 h of incubation. This figure is reproduced from [5] with permission. Copyright 2006 Elsevier.

over, various functionality and unique dimension and size of nanomaterials can enhance the bio-functionality to control the cellular behavior *in vitro* or *in vivo* by combining with the advantages of LbL assembly.

Mills and coworkers studied the effect of polymer multilayer films decorated with titanium dioxide (TiO_2) nanoparticles on the attachment, proliferation, and spreading behavior of mesenchymal stem cells (MSC) [51]. They showed that TiO_2 nanoparticles could be successfully assembled into the films by the alternating deposition with polyelectrolyte counterparts and the degree of attachment and spreading of MSC increases with the surface roughness, which could be controlled by the number of bilayers (Fig. 4). Moreover, the Kotov group has demonstrated that the LbL films consisting of single wall carbon nanotubes (SWNT) and poly(ethyleneimine) (PEI) could control the differentiation of mouse embryonic neural stem cells into different types of cells (i.e., neurons, astrocytes, and oligodendrocytes). Although the stage of stem cell study and the proposed mechanism require further investigation, it is scientifically meaningful to acknowledge that the LbL-assembled SWNT/polyelectrolyte composite films can induce the preferential differentiation into the specific lineage, giving us structural flexibility and the systematic tuning of physical and chemical properties of such composite films [52].

The patterning of polymer multilayer thin films based on the polymer-on-polymer stamping method is also recognized as a facile technique to program bio-functionality into the films. The cell-resistant surfaces can thus be patterned with varying densities of adhesion ligands or receptors to control the attachment of cells, leading to systematic study of the effects of ligand density on cellular functions. Rubner and coworkers generated cell adhesive patterns on cell resistant (PAA/PAAm) multilayer films by the polymer-on-polymer stamping with PAH crosslinked with RGD sequences, and demonstrated that the attachment and spreading of fibroblast cells increased as the RGD density of the patterns was increased [53]. Such cell adhesive patterns could also guide the cell alignment on such compliant LbL films including nanoscale control in film thickness, easy processing, and the ability to coat on various types of substrates. In addition, cellular co-cultures on patterned multilayer thin films are also available through the unconventional soft lithography as shown in Fig. 5 [54].

CONTROL OF CELLULAR FUNCTIONS THROUGH POLYMERIC MULTILAYERED FILMS

In biomaterial applications, polymer multilayer films are increasingly recognized as an influential tool to control key cellular functions [55,56]. Bioactive surface coatings can be readily incorporated on the multilayered films with different characteristics. As one example, a specific hormone, α-melanin-stimulating hormone, was conjugated to a polyelectrolyte and embedded in a polymer multilayer architecture in order to enhance the production of α-melanocortin by melanoma cells in bulk solution [57].

1. Control of Cellular Adhesion and Proliferation on Polymeric Multilayered Films

The behavior of cell attachment on polymeric multilayer films is

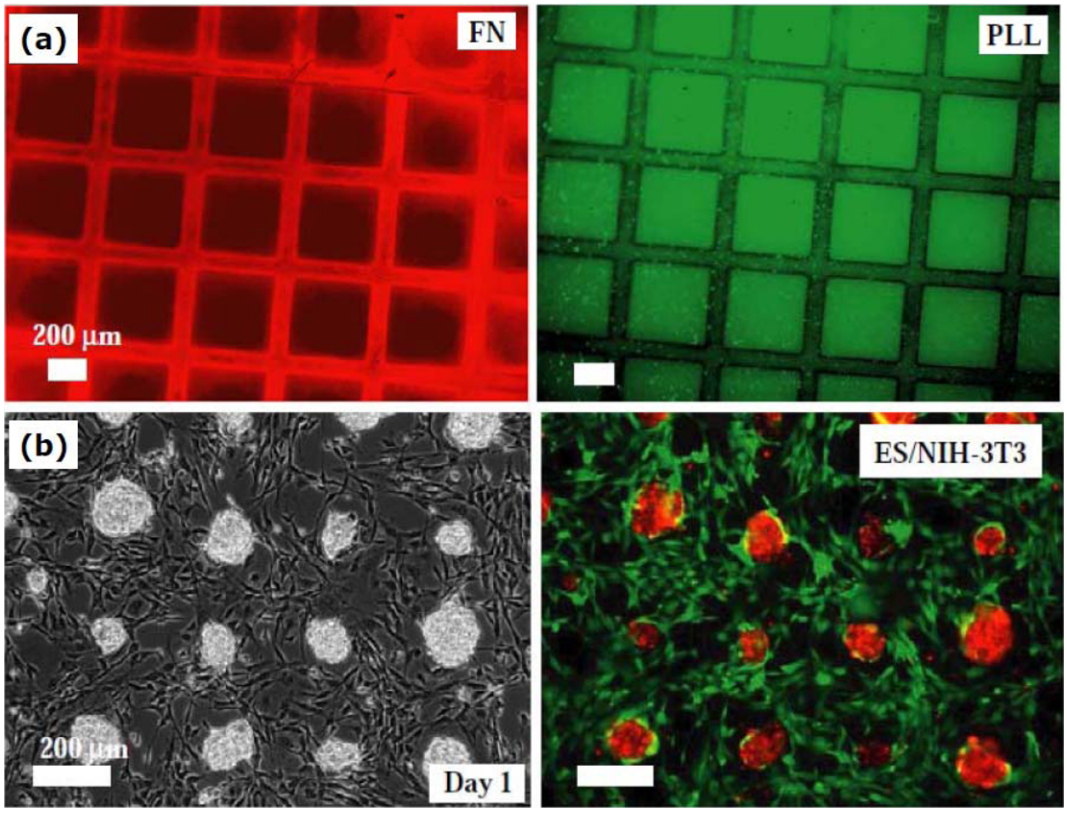

Fig. 5. (a) Fluorescent images of HA-patterned glass slides stained with FN (left) and subsequently treated with PLL (right) for co-cultures of ES cells with fibroblasts. (b) DIC (left) and fluorescent (right) images of patterned co-cultures of ES cells (red) with NIH-3T3 fibroblasts (green) after 1 day. This figure is reproduced from [54] with permission. Copyright 2003 Elsevier.

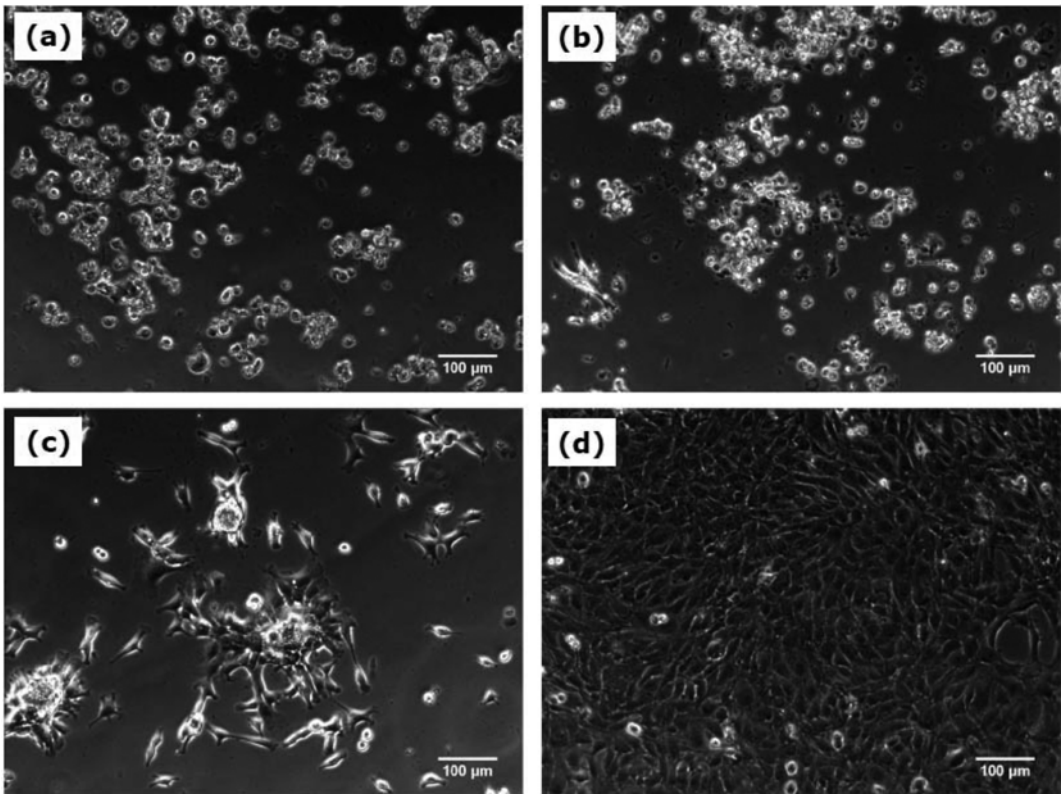

Fig. 6. Mouse myoblasts adhered onto (a), (b) a non-crosslinked film adopt a rounded shape, whereas the cells adhered onto (c), (d) photo-crosslinked films take the spreading shape. Moreover, in contrast to the cell shape on a photo-crosslinked film after a culture time of 15 h (c), an extensive proliferation of the cells was observed on a photo-crosslinked film cultured after 72 h (d). This figure is reproduced from [59] with permission. Copyright 2009 American Chemical Society.

the first phenomenon attributed to the contact of cells with a foreign body. Controlling the initial event of cell adhesion is one of the most critical steps influencing the cellular processes such as proliferation and differentiation [58]. More importantly, the surface modification by the LbL deposition has numerous possibilities to control the adhesion and proliferation of cells with multi-parametric functions that can be affected by various cues such as physical or chemical manipulations of the LbL films, surface topography (roughness, presence of nano- and micro-structures), and biological modifications, as have been mentioned previously.

We will, in this section, focus on several attempts to control the cellular adhesion and proliferation on polymeric multilayer films in more detail. Glinel and coworkers reported on the preparation of polyelectrolyte (PE) films based on biopolymers whose nanomechanical properties can be tuned by the photoinduced crosslinking [59]. Cell culture assays performed on non-crosslinked and photo-crosslinked films with myoblast cells demonstrated that the cell adhesion as well as proliferation was significantly improved with the increase in film rigidity (Fig. 6). Blacklock et al. reported another set of results based on the LbL films consisting of reducible hyperbranched poly(amide amine) (RHB) and DNA in order to investigate the fibroblast adhesion on $[RHB/DNA]_{n/2}$ films with varying rigidity. The surface modification of bioreducible LbL films of controlled film thickness and roughness promotes the activities of both

Table 1. Surface roughness, film thickness, the degree of swelling, and the surface zeta potential of various LbL films in PBS solution. This table is reproduced from [17] with permission. Copyright 2009 American Chemical Society

LbL films	Surface roughness (nm)	Film thickness (nm)	Degree of swelling[a] (%)	Zeta potential[b] (mV)
$(PAH/PAA)_9$	1.5	74.3	24.9	−30.3
$(PAH/PAA)_9$-X	0.8	52.7	3.3	−13.3
$(PAH/PAA)_9/PAH$	1.4	75.2	26.6	+69.8
$(PAH/PAA)_9/PAH$-X	0.7	53.1	2.1	+3.9
$(PAH/PAA)_9/(PAH/Fn)_2$	1.7	75.8	9.9	−26.0
$(PAH/PAA)_9$-X/$(PAH/Fn)_2$	1.0	53.8	4.9	−29.0

[a]Degree of swelling (%) = 100 × (wet film thickness − dry film thickness)/dry film thickness

[b]Surface zeta potential was measured in 10 mM NaCl buffer solution

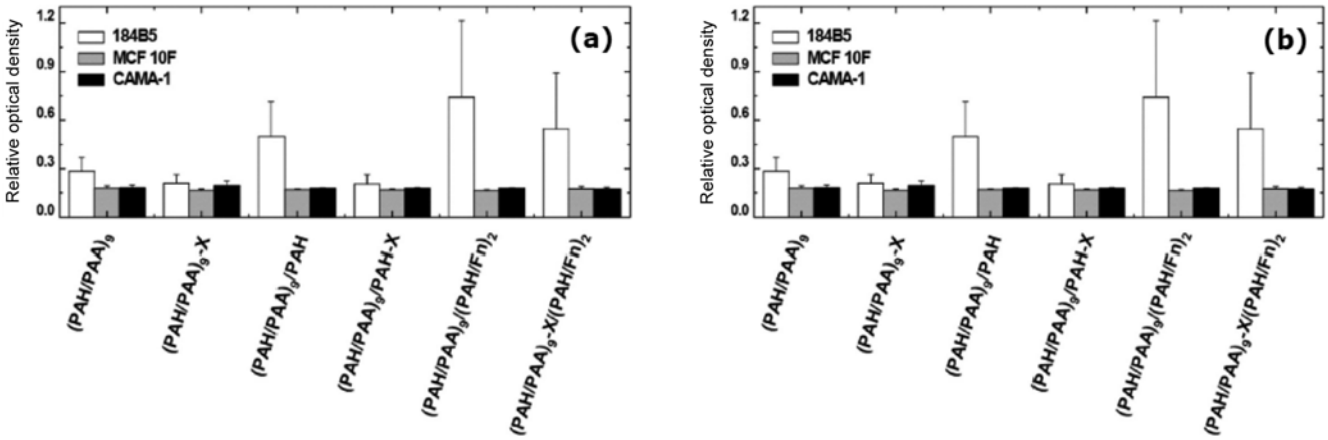

Fig. 7. (a) Relative cell number density (error bars: the standard deviations of measured optical densities from six replicate experiments) and (b) cell spreading area of normal 184B5, noncancerous fibrocystic MCF 10F, and metastatic cancerous CAMA-1 cells after 7 days of culture on various LbL films (error bars: the standard deviations of measured spreading areas/cells from four replicate experiments). This figure is reproduced from [17] with permission. Copyright 2009 American Chemical Society.

cellular adhesion and proliferation without additional coating of adhesive proteins on surface or crosslinking of the film [60].

As we have mentioned that the cell functions are multi-parametric events, various parameters related to the cell behavior should be investigated at the same time. In particular, surface properties such as charge density [21], hydrophilicity [61], and film rigidity [62,63] could be easily tailored by the adjustment of the chemical nature of polyelectrolytes, pH, temperature, number and/or thickness of layer pairs, and LbL deposition method. Char and coworkers evaluated the adhesion, proliferation, growth, and phenotypic change of three different human breast epithelial cell lines on spin-assisted multilayer LbL films [17]. Cellular phenotypic changes in response to various LbL films with different chemical and physical properties were analyzed by altering the terminal layer in contact with bulk culture solution, the rigidity of multilayer films, and the presence of cell adhesion promoting proteins (fibronectin) (Table 1 and Fig. 7). It has been shown that depending on the type of cells (normal, non-cancerous fibrocystic disease, and metastatic cancer cells), the external chemical and physical cues for the cellular phenotypic change are quite different, proving that various parameters in the multilayered films could significantly affect the behavior of specific cells and need to be carefully tuned unlike previous studies [64].

2. Control of Stem Cell Differentiation on Polymeric Multilayered Films

Stem cells have been recognized as an important source for cell transplantation since their distinct features involve the self-renewing capacity without restriction and the multilineage differentiation potential. For many years, significant efforts have been devoted to the development of protocols for the differentiation of neuronal cell types, insulin-producing cells, cardiomyocytes, or adipocytes from stem cells [65-68]. In fact, research on the stem cell differentiation in direct contact with polymeric multilayered films has just begun to flourish due to tunable features of polymeric multilayer films and, more specifically, the possibility of embedding many important elements affecting the cell destiny at desired locations within the films. We present here the yield of the stem cell differentiation to an intended lineage that could be controlled by various parameters such as *growth factors* and *film stiffness*.

Benkirane-Jessel and coworkers have demonstrated polyelectrolyte multilayer films that could drive embryoid bodies (EB) to cartilage and bone differentiation [69]. They prepared multilayer films constructed from the combinations of poly(L-glutamic acid), poly(L-lysine), and poly(L-lysine succinylated) within which transforming growth factor β_1 (TGFβ_1) and bone morphogenetic proteins (BMPs) have been embedded. Both BMPs and TGFβ_1 are required to be present simultaneously in the film for the bone differentiation, presumably due to the synergistic effect of the two active compounds. Based on these results, they also proposed the methodology which can induce or inhibit the cell death and control the tooth cell differentiation during tooth development [70].

Besides the growth factors, the mechanical properties of the multilayer films have been shown to play an important role in the higher order cellular differentiation. This view carries weight with us by two examples. The first example is the differentiation of breast epithelial cells into tubules when cultured in floating 3D collagen gels but no differentiation when cultured on gels attached to a culture dish [71]. The second is the outcomes that polyacrylamide gels of controlled stiffness could direct the specific lineage of stem cells. Soft platforms mimicking brain tissues are neurogenic while stiffer films mimicking muscles are myogenic. Also, comparatively rigid platforms similar to collagenous bones prove to be osteogenic [72]. An important question arises whether mechanical properties of polymer multilayer films could affect the cell differentiation without growth factors inserted into the film.

In Picart's work [73], multilayer films constructed from a pair of PLL and HA with controlled stiffness have been used to investigate the effect of mechanical properties of thin films on the skeletal muscle cell differentiation. Based on the carbodiimide chemistry, they varied the Young's moduli of multilayer films in the wide range simply by varying the crosslinker concentration. They qualitatively observed the cell differentiation such as cell alignment, fusion, and the formation of myotubes on various types of films and noticed important difference in the fate of the cells as well as in the kinetics of the events. In the case of soft films, only a few cells fused and

the cells began to detach only after a few days in a differentiation medium. In other words, cells progressively formed large aggregates or 'clumps' and started to lose connectivity with the films. This detachment phenomenon was quite delayed for stiffer films, presumably with the enhanced connectivity of the cells against the film surface.

3. Coating of LbL Multilayer Films on Cell Surface and Its Effect on Cellular Functions

Recently, different effects of LbL films coated on (multistacked) cells have been discussed in detail. Several researchers have reported the design of LbL films coated on the surfaces of red blood cells [74], platelets [75], yeasts [76], *E. coli* [77], and pancreatic islets [78].

Here, we would like to introduce the effect of various LbL films on common and basic cellular functions in order to clarify the universal effect of LbL films deposited on cell surface. Akashi and coworkers reported the effects of different types of LbL films placed on cell membranes on cell functions, focusing on the effects of charge, film thickness, composition, and morphology of the LbL films. Furthermore, they suggested a hierarchical cell manipulation technique for developing three-dimensional cellular multilayers by the deposition of LbL nanofilms, based on fibronectin (FN) and gelatin (G), on cell monolayers [79]. The placement of an FN-G multilayered film on the surface of the first cell monolayer provides a suitable cell adhesive environment, similar to the natural extracellular matrix (ECM), for the second layer of cells, thus realizing the three-dimensional cellular environment as shown in Fig. 8. A few years later, the same authors demonstrated that the component, charge, and the morphology of LbL multilayer films deposited directly on the surfaces of mouse L929 fibroblast cells strongly affected cell functions such as viability, morphology, and proliferation [80]. These results indicate that the LbL films topographically deposited on cell surfaces have vastly different results in cellular functions when compared to the PE films prepared on a substate. In particular, cells containing FN-G-dextran sulfate multilayer films with the FN-binding domains directly interacting with the cell surfaces demonstrated good cell proliferation profiles independent of film thickness, but PE films, in contrast, could not show such proliferation although the cells survived during the culture period.

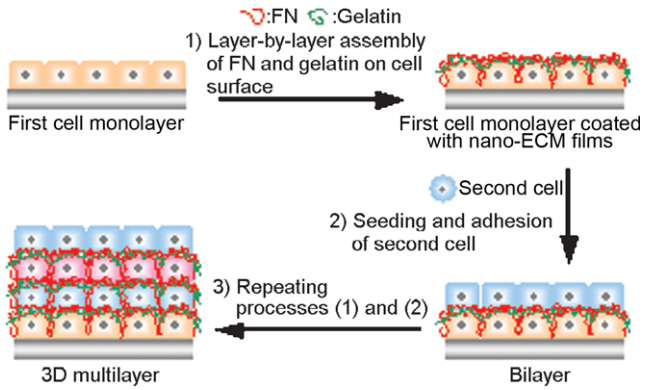

Fig. 8. *In vitro* fabrication of cellular multilayers by depositing a FN-gelatin film on the surface of each cell layer. The FN-gelatin film acts as a suitable cell adhesive surface similar to natural ECM. This figure is reproduced from [79] with permission. Copyright 2007 Wiley-VCH Verlag GmbH & Co. KGaA.

NOVEL BIOMEDICAL PLATFORMS BASED ON FUNCTIONAL POLYMERIC MULTILAYERED FILMS

Based on diverse methods controlling cellular functions by bio-functionalized polymeric multilayered films, novel biomedical platforms have been consistently suggested. One of the typical and commonly used platforms is the biocidal film coating with antibacterial or antifungal properties. The control of bacterial adhesion is easily achieved by tuning the physicochemical properties of polymer multilayer films containing natural antibacterial materials. Antibacterial peptides such as chromofungin [38] and gramicidin A [81] or Ag nanoparticles [82] have been successfully incorporated into the polymer multilayer films by the LbL deposition and these functionalized thin films clearly showed the biocial activity. Such antibacterial coatings have been widely used for biomedical devices to prevent chronic infections.

Another novel and promising platform based on the bio-functionalized LbL films is the bioactive coating to control human blood coagulation, which could have implications in drug eluting stents. Anticoagulation properties can be realized by decreasing the platelet adhesion to artificial surfaces. As a result, many researchers have focused on different LbL platforms to prevent platelet adhesion by controlling the wetting behavior, changing types or components of building block materials, or incorporating bioactive drugs. In most cases, charged biomacromolecules such as dextran (Dex), hyaluronan (HA) and chitosan (Chi) can be used for anti-thrombogenicity due to their intrinsic bioactivity. Akashi and coworkers reported the (Chi/Dex) LbL films with alternative anticoagulation and procoagulation bioactivity by varying salt concentration as well as the assembly step numbers [83]. Tabrizian et al. developed the (HA/Chi) multilayer films incorporating anticoagulants, nitric-oxide-donor sodium nitroprusside (SNP), within the films for drug eluting endovascular stents [84].

Furthermore, sustained and controlled drug release platforms based on stimuli-responsive polymer multilayer films have been developed to advance in the area of biomedicine and therapeutics, allowing for enhanced targeting, improved pharmacokinetics, lower toxicity, and improved patient convenience. One example of the controlled release platforms is hydrolytically degradable LbL thin films constructed from alternately depositing a degradable poly(β-amino ester) and a series of model therapeutic polysaccharides (i.e., heparin and chondroitin sulfate) [32]. These films exhibit pH-dependent and pseudo-first-order degradation/release behavior and have an exciting potential for the controlled release of a wide spectra of therapeutics [32].

In the context of controlled release, the procedure to prepare LbL multilayered films offers potential advantages over conventional protein and nucleic acid encapsulation strategies. For instance, enhanced gene transfer can be achieved by maintaining the elevated concentration of genes within relevant polymeric multilayer film carriers, which, in turn, would facilitate the gene introduction to cellular microenvironment. For instance, a multilayer film incorporating plasmid DNA, placed on a transparent indium-tin oxide electrode, was developed for spatial and temporal gene transfer through

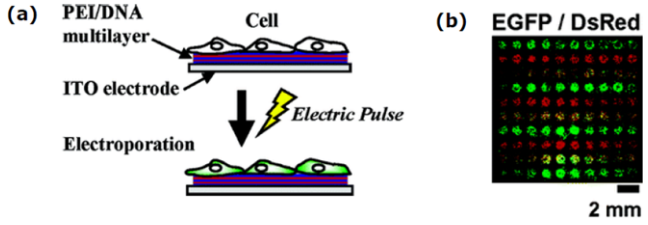

Fig. 9. (a) A schematic on (PEI/plasmid DNA) multilayer films onto transparent ITO electrodes for temporally and spatially specific gene transfer. (b) The electric pulse-triggered parallel gene transfection of HEK293 cells on a plasmid-arrayed ITO electrode. This figure is reproduced from [85] with permission. Copyright 2005 American Chemical Society.

the film (Fig. 9). Iwata et al. showed that cells were cultured on an electrode containing a multilayered film loaded with plasmid DNA and a short electric pulse was then applied to the cell/electrode system, followed by the verification of the release and transfection of plasmid DNA into primary cultured cells [85]. Many interesting reviews on the gene delivery system based on LbL multilayer films have already been published and readers are referred to these excellent reviews [86,87].

More recently, three-dimensional (3D) scaffolds controlling cellular functions have gathered keen interest because tissue organization relies on a complex 3D structure of cells and ECM moieties. Hence, there is a growing need toward constructing functional 3D scaffolds for building complex cellular architecture and studying realistic *in vitro* cellular functions. Particularly, controlling the cell adhesion and differentiation in 3D scaffolds is a considerable challenge in itself for tissue engineering applications [88]. We hope that this approach to construct functional 3D structures based on the LbL assembly provides a unique culture system for addressing critical questions in cell and developmental biology and also elucidates the potential mechanism for creating viable human tissue structures for therapeutic applications.

SUMMARY

Novel biomedical platforms ranging from antibacterial surfaces to three-dimensional scaffolds can be constructed by the LbL assembly based on diverse intermolecular interactions. In the context of LbL assembly, we have reviewed basic building-up principles employing different driving forces and deposition methods as well as process variables such as pH, ionic strength, light, heat, or enzyme to tune the physicochemical properties of thin multilayered films as well as the release behavior of such LbL films. A number of possible strategies to tune the properties of multilayer films can be further expanded to adjust cellular functions, particularly through the adsorption of proteins and specific ligands on such polymeric multilayered films. In addition, multilayer films prepared solely from natural biomaterials or biofunctional surface topography realized on such thin films can also be an effective biofunctionalization strategy to realize polymeric thin films more compatible for biomedical applications. The control of cellular functions such as adhesion, proliferation, and differentiation has been well documented based on the biofunctionalized multilayer films. Since cellular functions are typically known as multiparametric events, we have also noticed that a number of experimental parameters such as hydrophilicity, charge density, film rigidity, and the presence of active ingredients should be investigated in more systematic way to study the cell behavior in quantitative manner. We hope that this review, based on the functionalization of polymeric multilayer thin films, would serve as a reference for cell culture systems for targeted biological study and also give insights into the development of versatile biomedical platforms.

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Kookheon Char is a professor and the director of National Creative Research Initiative Program for Intelligent Hybrids Research Center in the School of Chemical and Biological Engineering at Seoul National University. He received his B.S. degree at Seoul National University, M.S. degree at KAIST and Ph.D. degree at Stanford University in US, taking all his academic degrees in Chemical Engineering. He has years in working at IBM Almaden Research Center and Lucky R & D Center in his capacity as a senior scientist before joining Seoul National University. He has been appointed as an invited professor at Ecole Superieure de Physique et Chimie Industrielle (ESPCI) in France as well as Massachusetts Institute of Technology (MIT) in US. He was also a visiting professor at Cornell University and Massachusetts Institute of Technology (MIT). His research focuses on the polymer functional platform based on *layer-by-layer* deposition methods and block copolymer templates. Furthermore, he is intensely interested in organic/inorganic nano-hybrids for energy/environmental applications and unconventional patterning for biological and optoelectronic applications.