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Polyelectrolyte Multilayer Assemblies on Materials Surfaces: From Cell Adhesion to Tissue Engineering

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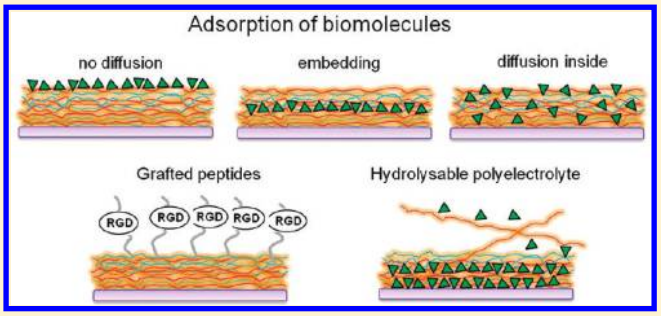
ABSTRACT: Controlling the bulk and surface properties of materials is a real challenge for bioengineers working in the fields of biomaterials, tissue engineering and biophysics. The layer-by-layer (LbL) deposition method, introduced 20 years ago, consists in the alternate adsorption of polyelectrolytes that self-organize on the material's surface, leading to the formation of polyelectrolyte multilayer (PEM) films.[1] Because of its simplicity and versatility, the procedure has led to considerable developments of biological applications within the past 5 years. In this review, we focus our attention on the design of PEM films as surface coatings for applications in the field of biomaterials, in tissue engineering, and for fundamental biophysical studies. This will include a survey of the chemical and physical properties that have emerged as being key points in relation to biological processes. The numerous possibilities for adjusting the chemical, physical, and mechanical properties of PEM films have fostered studies on the influence of these parameters on cellular behaviors. Importantly, PEM have emerged as a powerful tool for the immobilization of biomolecules with preserved bioactivity.



KEYWORDS: *layer-by-layer, polysaccharides, growth factors, cell adhesion cell differentiation, biomaterials, regenerative medicine*

1. INTRODUCTION

Controlling the bulk and surface properties of materials is a real challenge for bioengineers working in the fields of biomaterials, tissue engineering, and biophysics.

In the field of implantable biomaterials, the bulk properties of materials are known to be important for the overall properties, especially for mechanical strength, but their surface properties have long been recognized as being of utmost importance.[2] The surface of the material is an interface between the material and the host tissue, and it is able to trigger a wide variety of processes, from the initial inflammatory reaction to ultimate tissue remodeling. Considerable efforts are thus devoted toward functionalization of the surfaces of biomaterials used in biomedical applications (typically metals, polymers, ceramics) in order to render them bioactive, that is, able to trigger a specific cell response.[3] Polymeric coatings appear especially interesting because of the diversity of the chemical and physical properties they offer. For instance, polymeric coatings have been employed for the coating of stents.[4] Natural biopolymers appear promising as biomimetic coatings, as a result of their natural similarity to human tissues. A lot of effort is thus dedicated to engineering new forms of biomimetic surfaces. Tissue engineering has grown as a field in its own: its aim is to use a combination of cells, engineering, and materials and, together with suitable biochemical and physicochemical factors, improve the biological functions of damaged tissues (bone, cartilage, blood vessels, skin, etc.) or replace them.[5] Here, a scaffold in combination with cells and appropriate biochemical signals are needed to trigger a specific cell response and lead to formation of a new tissue. Synthetic polymeric materials can be employed as scaffolds when mechanical strength is needed, and hydrogels can be used for soft tissues.[6] Thus, surface modification of the scaffold may provide it with new functionalities.

Lastly, for more fundamental studies on cellular processes, biophysicists have already developed several tools to control the important properties of surfaces: the spatial presentation of the extracellular matrix proteins has been designed using micro-technologies to constrain cells in specific areas,[7] biochemical adhesive ligands have been grafted to surfaces in controlled amounts,[8] and more recently, the mechanical properties of the substrate have been recognized as playing a key role not only in cell adhesion[9] but also in cell fate.[10] Developments are now dedicated to the combined presentation of several stimuli and to the presentation of new types of biochemical ligands playing a role in cell fate, such as growth factors.[11] This would also help to investigate possible synergies between intracellular signaling pathways. Also, the presentation of biochemical stimuli is

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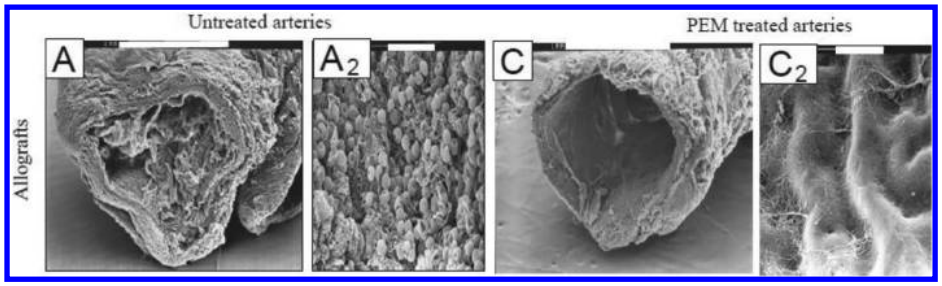

Figure 1. Scanning electron microscopy images of untreated and PEM-treated explanted arteries (A) were occluded and with pervasive thrombus (A2) after 1 week of implantation. When the artery was treated with a (PSS/PAH) film (C), the internal surface of the treated arteries (C2) showed no adherent cells and platelets. After 12 weeks, the treated internal artery surfaces (E2 and F2) showed a similar morphology to the native carotid internal surface (images not shown). Original magnification 400× (A and C) and 1000× (A2 and C2) (adapted with permission from ref 33, copyright 2008 Elsevier).

traditionally performed for cells grown on stiff substrates such as tissue culture polystyrene or glass. Now, the aim is to present them from softer, more physiological substrates.

Several techniques have thus been developed to design thin films at the molecular level, including Langmuir–Blodgett (LB) and self-assembled monolayers (SAMs). As already indicated by Kotov in his review,[12] both present a certain number of limitations and disadvantages. For biological applications, there was thus a need for easier and more versatile deposition methods. The layer-by-layer (LbL) deposition method, introduced by Moehwald, Decher, and Lvov 20 years ago, consists of the alternate adsorption of polyelectrolytes that self-organize on the material's surface, leading to the formation of polyelectrolyte multilayer (PEM) films.[1] The procedure is simple and versatile, as it is possible to control the processing parameters to modulate film growth and internal structure. Thus, the nature of the polyelectrolytes, the nature and number of the functional groups, the pH and ionic strength during assembly,[13] and the substrate used to build the films can be carefully chosen.[14] PEM film fabrication can be performed under mild conditions in an aqueous environment, which is a great advantage when using biopolymers and bioactive molecules. Film growth can be more or less rapid and films can either be stratified or exhibit some interdiffusion, which makes it possible to use them as either barriers[15] or compartments for the loading of bioactive molecules.[16]

Importantly, as will be shown below, PEM films appear highly suitable for immobilization of biomolecules with preserved bioactivity. They have emerged as a new type of coating, besides the more traditionally employed self-assembled monolayers and Langmuir–Blodgett films.[17] In fact, the most problematic disadvantage are probably the limited amounts of biological molecules incorporated into Langmuir–Blodgett films due to their limited stability. For self-assembled monolayers, there is a need for the presence of thiols on the substrate (e.g., for only noble metals or silane) in order to deposit them. For PEM films, no expensive equipment is required. In addition, surfaces of various chemistry and shape have already been coated with PEM films.[18] As will be shown below, one of the great advantages of the PEM technology is its ability to preserve the bioactivity of biological molecules and the possibility to deliver large amounts of biomolecules.

For all these reasons, there have been considerable developments in the past 5 years in the field of PEM for biomedical applications. Several reviews that include the biological field have been published. They concern either the internal structure of the films[14,19] or the applications of PEM films at the nanoscale.[20] These applications can be for controlled erosion using biodegradable polymers,[21] protein inspired nanofilms,[22] biosensors and biomimetics,[12] and drug delivery.[23,24]

In this review, we focus our attention on the design of PEM films as surface coatings for applications in the field of biomaterials, in tissue engineering, and for fundamental biophysical studies. This will include a survey of the chemical and physical properties that have emerged as being key points in relation to biological processes. The numerous possibilities for adjusting the chemical, physical, and mechanical properties of PEM films have fostered studies on the influence of these parameters on cellular behaviors.

We will include the different possibilities for controlling cell behavior by means of film composition, presentation of bioactive molecules, and modulation of mechanical properties. We will focus here on processes that require cell adhesion and will not review the potentiality offered by PEM films in cell transfection[25] or as antimicrobial coatings.[26] Also, because of limited space, we will not review other interesting aspects of controlling cellular processes through spatial organization of cell adhesion (i.e., nano or micropatterning). Spatiotemporal control offers other possibilities[18] that may open up new applications for PEM films that will be presented below.

Through selected examples from the literature, we will show that PEM films have truly emerged as a promising tool for the confinement of bioactive molecules, while preserving their bioactivity and delivering them locally. Very interestingly, these can be achieved via specific noncovalent interactions. By combining the different types of properties of PEM films, it is thus possible to control the early steps in cell adhesion but also longer time scale processes such as cell differentiation and tissue formation. Last but not least, the controlled presentation of bioactive molecules to cells by means of the engineered PEM films offers a new tool for biophysicists who are interested in unraveling the subtle interplay among cell adhesion receptors, growth factor receptors, and mechanotransduction pathways.

2. ROLE OF FILM COMPOSITION

2.1. Films Made of Synthetic Polymers. *2.1.1. Case of (PSS/PAH) Films.* Synthetic polyelectrolytes such as poly(styrene sulfonate) (PSS, a strong polyelectrolyte), poly(acrylic acid) (PAA), or poly(allylamine hydrochloride) (PAH) have been widely used in cell/film studies. The main advantage of using synthetic polymers is the possibility of adjusting certain parameters, including ionic strength and pH of assembly, to a considerable degree. Furthermore, they are easy to modify

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chemically. In this case, initial cell adhesion is mostly mediated through electrostatic interaction (i.e., nonspecific) and, more indirectly, via serum proteins adsorbed onto the films.

The most frequently studied synthetic PEM is, by far, linearly growing and dense (PSS/PAH) film. Its thickness can be precisely varied from a few nanometers to a few tens of nanometers.[27] Cell types such as endothelial cells,[28] fibroblasts,[29] osteoblastic cells,[30] and hepatocytes[31] have been cultured on these films. As a general rule, adhesion and proliferation on these films are very good. This may be attributed partly to the presence of sulfonate groups. (PSS/PAH) can be coated on the inner side of cryopreserved arteries[32] (Figure 1). This improved the mechanical properties of the cryopreserved vessel. It also made possible the adhesion and spreading of endothelial cells so that the internal structure of the vessel resembles that of fresh arteries. By looking at the expression of specific endothelial markers, namely, PECAM-1 and von-Willebrand-factor (vWF), the authors proved that the phenotype of the endothelial cells was preserved. In a subsequent study of the same group, PEM-treated arteries (rabbit carotids) as grafts bypassed native (untreated) rabbit carotids.[33] The *in vivo* evaluation of cryopreserved human umbilical arteries treated with (PSS/PAH) multilayers demonstrated a high graft patency after 3 months of implantation. Such modified arteries could constitute a useful option for small vascular replacement.

In another study, the same group investigated the differentiation potential of endothelial progenitor cells (EPCs), which are currently seen as very promising cells in tissue engineering for the design of autologous vascular grafts. Very interestingly, a rapid differentiation of endothelial progenitor cells (EPCs) into confluent mature endothelial cells was observed on (PSS/PAH) multilayers, which was higher than on conventional surfaces.[34] Indeed, the time needed to obtain these mature cells was reduced from two months to two weeks. Human mesenchymal stem cells (MSCs) were also found to differentiate into endothelial-like cells after only two weeks of culture on the (PSS/PAH) films, as shown by the expression of PECAM-1 and vWF. Thus, (PSS/PAH) films appear to have potential for vascular tissue engineering.

Also using human MSCs, Guillaume-Gentil et al. showed that (PSS/PAH) films on conductive indium tin oxide (ITO) electrodes can be used as a platform for growing viable cell sheets.[35] In this study, films made of nine layer pairs ending with PSS and of ∼20 nm in thickness were used. The resulting stem cell sheets retained their phenotypic profile and mesodermal differentiation potency. The authors showed that both an electrochemically induced local pH lowering and a global decrease in the environmental pH resulted in a rapid detachment of intact stem cell sheets. Furthermore, they evidenced that the recovered stem cells sheets maintained their capacity to differentiate toward the adipogenic and osteogenic lineages.

2.1.2. Other PEM Films Made of Synthetic Polyelectrolytes. Another type of popular PEM assembly is the (PAA/PAH) system, which was initially developed by Rubner's group.[36] The thickness of these films can be varied by changing the pH of the assembly.[13] Interestingly, the topography of such films can be modified by post-treatment in acidic solution to render them either nanoporous or submicroporous.[36] Rajagopalan et al.[37] investigated the potential of such films for wound healing in the cornea, using corneal epithelial cells as cellular models, as the epithelium presents a physical barrier to external agents. During wound healing, corneal epithelial cells undergo proliferation and migrate to the wound site. In their study, they created pore diameters in the 100−600 nm range by post-treatment of (PAH/PAA) films in solutions of pH ranging from 1.9 to 2.5. Porous surfaces that exhibited either 100 nm (nanoporous) or 600 nm (submicron) pore diameters supported corneal cell adhesion, but the nanoscale porosity significantly enhanced corneal epithelial cellular response. Corneal epithelial cell proliferation and migration speeds were significantly higher on nanoporous topographies. The actin cytoskeletal organization was well-defined, and vinculin focal adhesions were found in cells presented with a nanoscale environment. These trends prevailed for fibronectin (FN)-coated surfaces as well, suggesting that, for human corneal epithelial cells, the physical environment plays a defining role in guiding cell behavior. Of note, FN is a cell-surface protein, which is a very important component of the extra-cellular matrix (ECM), as it mediates cellular adhesive interactions.

2.2. Films Made of Natural Polymers. Tissues are composed of cells embedded within an ECM made of proteins, polysaccharides, and other bioactive molecules such as growth factors. ECM provides the cells with mechanical and biochemical signals.

ECM proteins, polysaccharides, or their fragments can be used in PEM construction to promote cell adhesion and proliferation. Entire films can be made of ECM components; ECM molecules or their fragments can be adsorbed on the film's surface or covalently linked, respectively. A step closer to recreating the original matrix, in which cells develop *in vivo*, is to use ECM components as building blocks for the films.

Natural biopolymers, such as collagen (COL),[38,39] gelatin,[40,41] hyaluronan (HA),[42] chondroitine sulfate (CS),[43,44] or heparin (HEP)[45] can be used as building blocks for PEM films. This type of PEM film provides compositional uniqueness, such as stimulating a specific cellular response, and serves as both mechanical and biochemical signals.

Type I collagen is a major protein component of fibrous connective tissues, which provides mechanical support and frameworks for the other tissues in the body. Collagen is a natural ligand for several cell receptors in the integrin family. Gelatin is a partially hydrolyzed and denaturated form of collagen.

HA, CS, and HEP belong to the family of glycosaminoglycans, which are made of disaccharide repeating units containing a derivative of an amino sugar, either glucosamine or galactosamine. They contain negatively charged carboxylate and/or sulfate groups. HA and CS are responsible for the unique hydration and mechanical properties of synovial fluid, cartilage, and tendons. HA and CS are highly hydrated polymers surrounded by respectively ∼20 and ∼30 water molecules per disaccharide unit in interaction through hydrogen bonds.[46] Importantly, these polysaccharides are part of the pericellular coat (also called glycocalyx). This coat, which can be up to several μm in thickness,[47] plays a major role in the interactions between a cell and its environment by mediating cellular adhesion and the diffusion of biomacromolecules such as growth factors.[48] HEP has several possibilities for sulfate groups. Some forms act as anticoagulants by binding specifically to antithrombin, which accelerates the sequestration of thrombin. This is why HEP is often used as an anticoagulant.

In an early study, gelatin was associated with polyethyleneimine (PEI) and deposited on a synthetic degradable poly(DL-lactide) substrate.[49] Chondrocytes were found to attach and

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proliferate, and their viability was good on these PEM-modified scaffolds.

Gelatin was also associated with chitosan (CHI), a polysaccharide that is not present in the human body but can be found in crustacean shells, and deposited onto titanium films.[41] Here, the authors showed that the proliferation and viability of osteoblast cells on the PEM-modified titanium substrates were better than on control surfaces after 1 day and 7 days of culture *in vitro*.

COL has been associated with HA to build (COL/HA) films.[39] Interestingly, the fibrillar structure of collagen was preserved, as observed by AFM imaging of the films. The authors showed that chondrosarcoma cells spread well and synthesized the extracellular matrix components solely on the collagen ending films, whereas no cellular matrix was found for those ending with HA.

The introduction of HEP into PEM films is often applied to coatings of blood-contacting biomaterials. In fact, endothelialization and antithrombogenicity are two key issues in stent implantation. Heparin was initially introduced with PEI.[50] (COL/HEP) films have been employed as titanium coatings to study endothelial progenitor cell (EPC) attachment and proliferation.[51] *In vitro*, the (COL/HEP) greatly increased EPC attachment and proliferation, as only 3 days were required to form a confluent layer. Furthermore, platelet adhesion was found to be reduced on such coatings.

As CS is an important component of cartilage and bone tissues, the adhesion of bone cells to (CS/HEP) coatings was investigated.[52] When CS was used as a film component, the films displayed a low Young's modulus and cell adhesion was poor. However, the cells responded differently when CS was adsorbed onto a stiffer polypeptide PEM basis. Similar films made of (COL/HEP) and (COL/CS) were built on poly(dimethylsiloxane) (PDMS) substrates,[53] with COL retaining its fibrillar structure. Whereas (COL/CS) films were stable in culture medium, (COL/HEP) were not. Primary bovine chondrocytes were found to adhere better on PEM films than on tissue culture polystyrene. Interestingly, these authors showed that β1 integrin antibodies prevented cell spreading, suggesting that cell adhesion and spreading were specifically mediated by interactions with the collagen fibrils.

One advantage of these natural components is their ability to specifically interact with living cells, their bioavailability, and their possible biodegradability, as specific enzymes are present in tissue and biological fluids.

2.3. Role of the Final Layer: Surface Charge and Hydrophobicity. Because PEM films are 2D materials, not only their entire composition but also their surface composition is important. Surface charge can affect protein adsorption (depending on the pI of the protein) and ultimately cell adhesion. The typical functional groups of the polyelectrolytes are carboxylic acid, sulfate, sulfonate, as negatively charged groups, and amine, as positively charged groups. In the case of synthetic polyelectrolytes, PAA-ending films (carboxylic group) were found to be resistant to the adsorption of BSA, fibrinogen, or even to lysozyme, which is oppositely charged to PAA.[54] This was explained by the low charge density of PAA but also by its strong hydration, which creates an exclusion volume above the PAA layer. Usually, proteins adsorb preferentially onto films of opposite charge.[54,55] For instance, PAH-terminated films lead to a very high adsorption of proteins from the serum.[56] On PSS-ending films, certain serum proteins present in the cell culture medium, such as BSA, adsorb weakly[57] and may be implicated in the cell response to PSS-ending films.

However, it now seems to be accepted that protein adsorption alone cannot account for the significant differences in cell adhesion.

Depending on the cell type, cells may prefer positively or negatively charged film-ending layers. For instance, hepatocytes grown on the films made from synthetic polymers poly(dimethyldiallylammonium chloride) (PDDA) as polycation and PSS[58] adhered only to the films terminated with a PSS layer and not to PDDA-ending films. However, other cells lines, such as fibroblasts, were less sensitive and adhered on both the PDDA and PSS-ending films.

In a different field of application, the differentiation potential of myoblast into myotubes was assessed.[59] The authors first investigated the growth of myoblasts on PSS or PAH-ending films with PSS of different molecular weighs. They found better viability and growth on PSS-ending films but observed that there was no difference for PSS of different molecular weights. They also followed differentiation into myotubes over 7 days and observed that it was more effective on PSS-ending films, as assessed by the higher fusion index. The molecular weight of the PSS had no influence.

Hydrophilicity/hydrophobicity are also known to influence protein interactions with the surface and cell adhesion, as well. Synthetic polymers were employed by Salloum et al. for investigating the combined effects of increasing surface charge and hydrophobicity[60] on vascular smooth muscle cell (SMC) adhesion. On the most hydrophobic surfaces, the A7R75 SMCs spread and were not very motile. Conversely, on the most hydrophilic surfaces, these cells adhered poorly and displayed characteristics of being highly motile.

It was shown that surface wettability, surface charge, and lateral structures could be controlled by changing the pH value of the HEP solution to acidic, neutral, or alkaline values during the multilayer assembly of PEI and HEP multilayer films, resulting in modulation of fibroblast adhesion.[61] All terminal layers were cytophobic, unless preadsorption of serum or of FN was achieved. The effect of the serum was more prominent on PEI final layers, probably as a result of their positive surface charge, whereas the effect of FN was more pronounced on HEP terminated multilayers, possibly as a result of its ability to bind FN specifically. The PEM films that were initially nonadhesive were also found to inhibit fibroblast growth. On the contrary, those favoring cell adhesion also induced higher cell growth and metabolic activity.

To conclude, surface charge and hydrophobicity of PEM films can have a significant impact on cell adhesion, in a manner that depends on the nature of the functional groups and on the cell types, as well.

2.4. Influence of Film Thickness. Some films are known to grow linearly over a wide range of conditions, whereas other PEM films grow exponentially. This is the case for (PLL/PGA),[62] (PLL/HA),[63] and (PLL/CSA)[43] films built in physiological conditions (pH 7.4, 0.15 M NaCl). Film thickness is related to the ability of the polyelectrolyte to take up water, to the charge matching of the polyelectrolyte pairs, and to the affinity of the polyelectrolytes for each other.[14] Usually, PEM films made from highly hydrated polysaccharides and polyaminoacids yield gel-like films. In such cases, the films are very soft and hydrated, and cells adhere poorly to them.[64−66] On the contrary, as mentioned above (see section

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2.1.1.), cell adhesion is usually good on (PSS/PAH) films, exhibiting linear growth.

Film thickness can also be modulated by pH variations during film assembly, one of the best characterized PEM films in this category being (PAH/PAA) films. Rubner's group has plotted the film thickness matrix as a function of the pH of each polyelectrolyte solution,[13] with thick films being formed when the pH of PAA is close to 2, while highly stitched and dense films are formed at neutral pH. (PAH/PAA) films were found to be nonadhesive when films were built at pH 2 (thick films), whereas high adhesion was observed for films built at pH 6.5. This was attributed to the ability of the former films to swell.[67,68]

3. MODULATION OF FILM MECHANICAL PROPERTIES

It is increasingly accepted that cell processes depend on the reciprocal and dynamic interactions of cells with their surrounding microenvironment, which includes biochemical and mechanical stimuli defined by neighboring cells and extracellular matrices.[69] Cells are mechanosensors known to transduce a mechanical signal into a biochemical signal or vice versa. Specific proteins are known to play a key role in this process, and among those are integrins. Integrins are transmembrane receptors that exhibit conformational changes in response to mechanical stimuli.[70] Some components of the adhesive structures of the cells that are formed during adhesion (e.g., focal adhesions) can also exhibit conformational changes and transducer forces. Many cell types are sensitive to the mechanical properties of the underlying substrate and respond by increasing their adherence, spreading, and proliferation.

In a pioneer study by Discher's group, decoupling (or independent adjusting) of the mechanical and chemical properties has been achieved, using model synthetic gels such as polyacrylamide gels grafted with COL at increasing densities.[71] The same group showed that altering polyacrylamide gel stiffness made possible MSC differentiation into neurons on soft PA gels, bone cells on stiff gels that mimicked collagenous bone,[10] and myoblasts for gels of intermediate stiffness. Other types of synthetic and natural polymeric materials with controlled mechanical properties have been developed, such as poly(ethylene glycol),[72] PDMS, alginate,[73] or hyaluronan.[74]

Although a full decoupling of mechanical and chemical properties is the ideal goal, this is, in fact, very difficult to achieve. There are several reasons for this: First, many of the cross-linking strategies are based on a chemical modification of the material. Second, biochemical ligands are added by grafting it or by adsorbing it. Grafting involves a chemical modification at the surface of the material and adsorption, which relies on noncovalent interactions, and is a natural process that depends on the physicochemical properties of both the material and the protein.

PEM films are materials whose mechanical properties can be controlled in several ways, thus allowing cell studies on the films of different stiffnesses.

Different strategies employed for modulating the mechanical properties of PEM films have already been reviewed: adding "stiff" layers,[75] modulating pH during assembly,[76] chemical cross-linking by means of glutaraldehyde[77] or by means of a carbodiimide,[64] photocross-linking using photosensitive derivatives of the polyelectrolytes,[78,79] or incorporating nano-objects into the film.[80] See Figure 2.

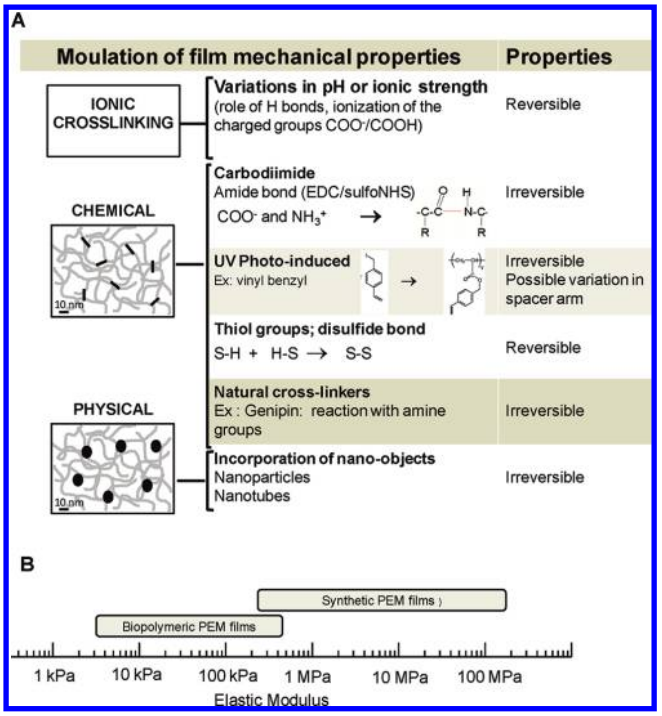

Figure 2. (A) Overview of the main strategies used to modulate mechanical properties of polyelectrolyte multilayer films. The methods are essentially based on ionic cross-linking, chemical cross-linking, and physical cross-linking. (B) Range of stiffnesses of the natural and synthetic polyelectrolyte multilayer films.

As mentioned by Sukhishvili et al.,[24] cross-linking is often applied to convert PEM films into "surface hydrogels". Often, soft and hydrated films do not exhibit stratification, but what is required here is rather a change in their "bulk" properties. The film is seen as a "surface adsorbed hydrogel" whose confinement provides it with very interesting properties for local changes in biochemical and mechanical properties. Of note, this modulation in mechanical properties over 1 or 2 orders of magnitude in Young's modulus can only be achieved if the film's stiffness in un-cross-linked conditions is sufficiently low.

3.1. Adding "Stiff" Layers. For instance, (PSS/PAH) films are already so stiff (Young's modulus of up to 100 MPa) that nobody has tried to stiffen them even more. Instead, they can be employed as a rigid barrier to stiffen (PLL/HA) films.[75,81] Thus, by adding several layer pairs of (PSS/PAH) onto the (PLL/HA) basis, Vautier et al.[81] have shown that the adhesion of kidney epithelial cells progressively increased when the films became stiffer. In a very elegant manner, they have studied the influence of substrate elasticity on replication and transcription, using such PEM films as model substrates. The sequential relationship between Rac1 (a very important protein involved in cytoskeletal changes), vinculin adhesion assembly, and replication becomes efficient at above 200 kPa because activation of Rac1 leads to vinculin assembly, actin fiber formation, and, subsequently, to the initiation of replication. Above 50 kPa, transcription was correlated with the engagement of a specific integrin (αv-integrin), together with histone H3 hyperacetylation and chromatin decondensation, allowing little cell spreading. In contrast, soft substrates (below 50 kPa) promoted morphological changes characteristic of apoptosis, including cell rounding, nucleus condensation, loss of focal

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adhesions, and exposure of phosphatidylserine at the outer cell surface.

3.2. Modulating pH during Assembly. As shown by Van Vliet et al., the stiffness of (PAH/PAA) films assembled at different pH can be varied from 200 kPa to 142 MPa and can affect cell function.[76] They showed that the adhesion and proliferation of human microvascular endothelial cells strongly increased as the PEM became stiffer.[76] In another work, the same group adjusted independently the mechanical and chemical properties of films by modifying the film's surface with COL I or a mixture of COL I/decorin and studying primary hepatocyte adhesion and functions. These cells are widely considered to be ideal for constructing liver tissue models but are known to rapidly (within a few hours or days) lose their viability and phenotype functions upon isolation from the native *in vivo* microenvironment of the liver. They found that, on unmodified (PAH/PAA) surfaces, hepatocyte attachment increased with PEM rigidity,[82] but this trend was canceled when the PEM substrata was modified with COL I or with COL I premixed with the small proteoglycan decorin. They also demonstrated that hepatic albumin secretion (a marker for liver-specific protein synthesis) over two weeks decreased with increasing substrata stiffness, indicating that hepatocytes formed stable, spheroid aggregates preferentially on protein-modified compliant surfaces, whereas cells detached from stiffer substrata after only a few days of culture. Such detachment was presumably due to the dominance of cell−cell over cell−substrata interactions.[82]

3.3. Chemical Cross-linking. Chemical cross-linking by means of carbodiimide chemistry has been applied to various PEM films and quantified by means of infrared spectroscopy and AFM (atomic force microscopy) nanoindentations.[83] Amine and carboxylic groups are converted into covalent amide bonds[64] in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). Chondrosarcomas, chondrocytes,[84] and osteoblasts,[66] were to adhere more when the (PLL/HA) or (PLL/PGA) film[66] stiffness is increased. For SMCs[85] and skeletal muscle cells,[86,18] an increase in cell spreading on (PLL/HA) cross-linked films as well as on (PAH/PGA) cross-linked films was observed.

The behavior of two different types of stem cell was investigated on (PLL/HA) cross-linked films: MSCs and embryonic stem cells (ESCs). MSCs represent a particularly interesting cell type for research and therapy because of their ability to differentiate into mesodermal lineage cells such as adipocytes, osteocytes, chondrocytes, cardiac muscle, or endothelial cells.[87] Zisch et al. observed that native (PLL/HA) (i.e., un-cross-linked) showed poor adhesion for MSCs despite a high surface density of preadsorbed FN.[88] However, MSC adhesion and proliferation was very good on cross-linked (PLL/HA) films. Covalent attachment of FN was necessary to maintain the MSC over weeks for their differentiation. Furthermore, the MSC were capable of differentiating into osteocytes and chondrocytes upon culture with induction factors.

The behavior of ESCs on (PLL/HA) films has also been investigated. ESCs are derived from the inner cell mass (ICM) of the blastocyst at an early stage of embryonic development, following the segregation of the embryo into the ICM and the trophectoderm.[89] Blin et al. showed that ESC adhesion and proliferation increased on stiffer films. ESCs were also shown to keep their pluripotency when grown on native nanofilms, which prevented their adhesion. Their phenotype was more reminiscent of the ICM stage of embryogenesis. Furthermore, cells grown on native (PLL/HA) films exhibited a better potential for differentiation than cells grown on cross-linked films. These latter cells reached the epiblast stage, which had a more limited repertoire of differentiation.

The chemistry of the native film played an important role in the maintenance of ESC pluripotency. In fact, the native films, but not cross-linked ones, released a small amount of PLL, which was sufficient to induce the expression of ICM genes by the ESC cells.

This very small release may be related to the mechanical properties of the native (PLL/HA) film. Indeed, reflection interference contrast microscopy and confocal laser scanning microscopy experiments have evidenced that native (PLL/HA) film is rather a viscoelastic liquid whose equilibrium elastic modulus is zero.[90] This was not observed for EDC-cross-linked films.

3.4. Photo-cross-linking. Photo-cross-linking is another way of modifying a film's mechanical properties after film buildup, provided that one of the polyelectrolytes has a photosensitive group.[78] Pozos-Vasquez et al. also reported on the preparation of polyelectrolyte films based on PLL and HA derivatives modified by photoreactive vinylbenzyl (VB) groups.[79] The VB-modified HA incorporated into the films by UV irradiation, and the force measurements taken by atomic force microscopy proved that the rigidity of the cross-linked films increased up to four times. Adhesion of myoblast cells increased on the stiffest films.

These research papers, studying different cell types on different PEM films, highlight on the one hand the strong dependence of cell processes on both the mechanical and chemical properties of the substrata and, on the other, the difficulties for decoupling these two distinct properties. Thus, care is needed when concluding from the respective roles of these factors that are often correlated.

3.5. Incorporating Nanoparticles. Incorporating nano-objects into an organic matrix is another way of stiffening it, and has already been widely applied to PEM films.[91,92] Different types of nano-objects have been introduced as film components, including carbon nanotubes[93,91] and montmorillonite;[94] evaluating the mechanical properties of these composite films displayed up to 2 orders of magnitude more on Young's modulus when compared with the pure polyelectrolyte.[95] The mechanical properties also depend on the nonaggregated or aggregated state of the nanoparticles.[96] Such composite assemblies with interesting mechanical and electrical properties appear particularly interesting for the coating of neuroprosthetic devices.[97] In a first study, Kotov et al. showed that thin PEM membranes containing single-walled carbon nanotubes (SWNT) supported extensive neurite outgrowth.[91] Later on, the same group demonstrated that mouse embryonic neural stem cells (NSCs) could be successfully differentiated into neurons, astrocytes, and oligodendrocytes with clear formation of neuritis on the PEI/SWNT multilayer films.[80] NSCs behaved similarly to those cultured on the standard and widely used poly(L-ornithine) substratum in terms of cell viability, development of neural processes, and appearance and progression of neural markers.

More recently, synthetic PEI was replaced by the protein laminin, which is an important component of the extracellular matrix of the brain, to "humanize" the carbon nanocomposite film.[98] The authors found that the adhesion of NSCs up to 7 days in culture depended on the outermost layer and on the

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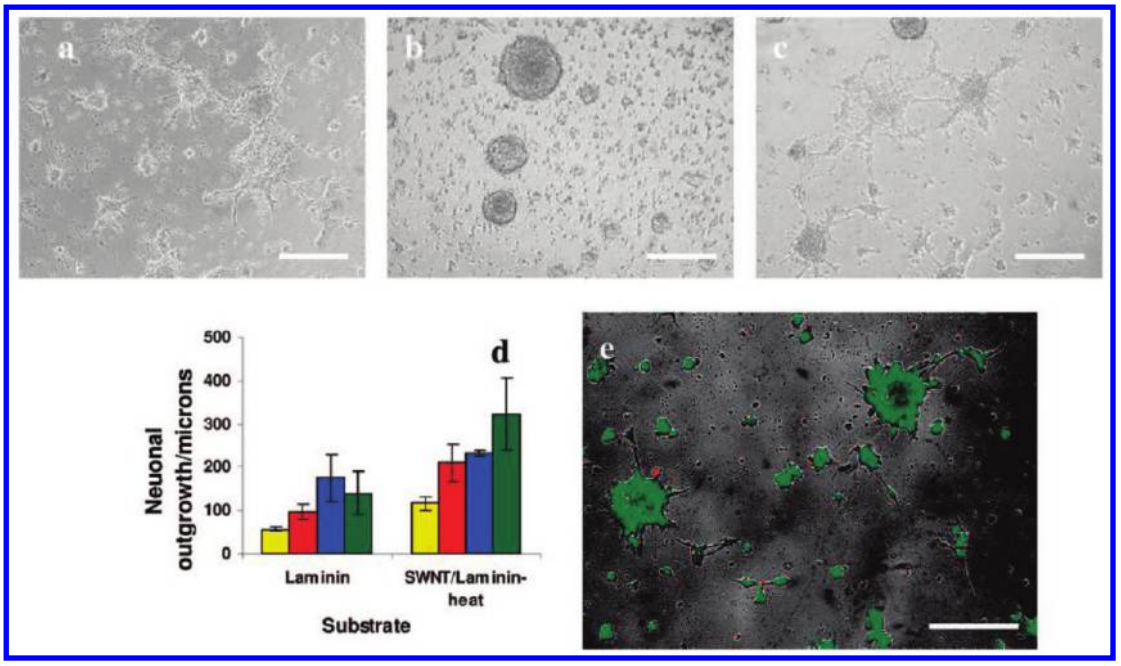

Figure 3. Micrograph assessing neural stem cell adhesion and differentiation 72 h after initial seeding on (a) laminin-coated glass slides and on 10 bilayer SWNT/laminin thin films that were (b) used as-is or (c) heated at 300 °C for 10 min. (d) Distance of outgrowth from neurospheres after 24 h (yellow), 48 h (red), 72 h (blue), and 120 h (green) on laminin-coated slides and heat-treated SWNT/laminin film on slide. (e) Live–dead viability assay on seeded cells where live cells are stained green and dead cells are red. Scale bars are 200 μm (reproduced with permission from ref 98, copyright 2009 American Chemical Society).

post-treatment (heating at 300 °C for a very short time) (Figure 3). The (SWNT/laminin) nanocomposites did not support cell adhesion unless they were stiffened by heating. The substrate that was most conducive to cell adhesion and attachment was the PEM film that contained SWNT as the topmost layer and that was heat treated. Extensive formation of functional neural networks was observed, as indicated by the presence of synaptic connections. Importantly, 98% of the cells were found to remain viable. Immuno-staining of specific neuronal markers MAP-2 (for neurons), glial fibrillary acidic protein, GFAP (for astrocytes), and nestin (for NSCs) was performed after 7 days of culture. Interestingly, it was found that differentiated neurons and glial cells were present in large amounts as a result of spontaneous differentiation caused by the physical properties of the SWNT/laminin composites. Furthermore, calcium imaging of the NSCs revealed generation of action potentials upon the application of a lateral current through the SWNT substrate. All together, these results appear very promising, as they indicate that the protein/SWNT composite can serve as the material foundation of neural electrodes with a chemical structure better adapted to long-term integration with the neural tissue

3.6. Modeling the Cell Response. There are still only a few models of cell interactions with PEM films. In a model of cell adhesive behavior on thin polyelectrolyte multilayers, Chan et al.[99] implemented a finite element analysis to help elucidate the trends observed in cell spreading, such as decreased cell spreading when the number of layer pairs in the film was increased (for very thin films of less than 100 nm in thickness). The authors correlated the focal adhesion area to the amount of work done by the cell during active mechanosensing. The film was modeled as a linear elastic material. An "effective stiffness" was defined to account not only for its mechanical properties but also for its thickness and for the number of focal adhesions recruited. Their results suggest that the energy consumed by the cells during active probing with a constant adhesion force regulates cell morphology and adhesion behavior.

Further modeling of cell/film interactions may help to better understand the role of various parameters in cell response.

4. BIOCHEMICAL FUNCTIONALIZATION

Biochemical functionalization can be achieved to activate a specific cellular signal. Presentation of a biochemical signal by the PEM films allows this signal to be spatially controlled at the cell adhesion site. The biochemical signal can also be potentially sustained for a long time period. Different strategies may be employed (Figure 4).

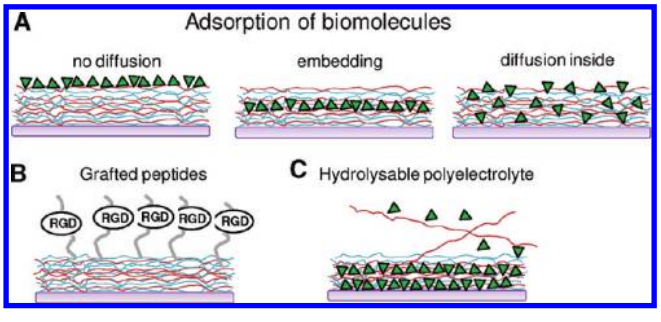

Figure 4. Scheme representing different possibilities of incorporing bioactive molecules inside or on top of PEM films. (A) Adsorption of the bioactive molecule can be achieved after film buildup or at a certain step during build up. In the case of diffusion, the bioactive molecule can be loaded in the "bulk" of the film. (B) Very small molecules such as bioactive peptides can be grafted to one of the polyelectrolytes. (C) If one of the components is hydrolyzable, then the bioactive molecule can be delivered in solution.

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4.1. Modification by ECM Molecules. *4.1.1. Adsorption of Entire ECM Proteins on PEM Films.* To improve cell adhesion on PEM films, adsorption of ECM proteins (Figure 4A) onto multilayers is a useful tool, usually achieved using COL or FN as adhesive proteins. These proteins are widely used by biomaterial scientists,[100] biophysicists,[71] and biologists,[101] as they are important ECM proteins and probably the best characterized. The aim is to provide specific attachment to the cells via integrin receptors.

Wittmer et al.[31] investigated the effect of various parameters on the attachment and function of three different types of hepatic cells, human hepatocellular carcinoma (HepG2) cells, rat hepatocytes, and human fetal hepatoblasts. To this end, they chose different types of PEM film, such as (PAH/PSS), (PLL/PGA), or (PLL/ALG) films, and systematically studied the influence of composition, terminal layer, and rigidity (using EDC cross-linked films; see section 3.3). They also investigated the influence of a terminal layer of COL I or COL IV, a prominent extracellular matrix protein within the human liver parenchyma. In a first step, they studied cell attachment and growth over a 7-day period, and in a second step, they quantified albumin secretion of the confluent systems. Importantly, all the PEM systems produced albumin, indicating the presence of functional hepatoblasts and/or hepatocytes. The cross-linked $(PLL/PGA)_n$-PLL films promoted the greatest level of function at 8 days. From the large set of conditions they studied, they conclude that film composition, terminal layer, and rigidity are key variables in promoting attachment and function of hepatic cells, while film charge and biofunctionality were somewhat less important.

Using (PAH/PAA) films as a matrix for hepatocyte attachment, Van Vliet et al. showed that adsorption of COL on the softest films (PEM built at pH 2) led to enhanced hepatocyte attachment, which was statistically similar to the stiffer, unmodified, and protein-modified substrata.[82]

The effect of FN adsorption onto PEM films has also been investigated in some specific cases. For instance, Olenych et al.[68] found that FN bound best to PAH-terminated and Nafion-terminated PEMs but poorly to PEM films terminated with a copolymer of PAA. A7r5 smooth muscle cells were found to adhere and spread well on the Nafion-terminated PEM surfaces. In contrast, cells spread less and migrated more on both FN-coated and uncoated PAH-terminated PEM surfaces. Interestingly, these results indicate that A7r5 cell adhesion, spreading, and motility on PEMs can be independent of FN binding to the surfaces.

Using (PLL/dextran sulfate) PEM films, Wittmer et al.[102] also showed, by means of quantitative measurements of FN adsorption, that FN adsorption on PLL-terminated films exceeded that on dextran sulfate-terminated films by 40%, correlating with the positive charge and lower degree of hydration of PLL-terminated films. They followed the attachment of endothelial cells (human umbilical vein endothelial cells) and found that PLL-ending films exhibited a greater extent of cell spreading than dextran sulfate-ending films. Furthermore, adsorption of FN led to an increase in cell spreading. For these PEM films, they concluded that the presence of FN was an important factor, more than film charge or layer number, in controlling the interaction between multilayer films and living cells.

On the basis of all these studies, it appears that it is not possible to draw a unique conclusion. In some cases, film composition and mechanical properties can be more important than the biochemical signal provided by the adsorbed ECM molecules. However, in the case of poorly adhesive films (especially soft PEM films), the biochemical signal may compensate and lead to engagement of integrin cell receptors, leading to increased cell attachment and spreading.

It should be noted that solely quantifying the adsorbed amount of protein is not predictive enough of the conformation of the protein on the PEM films. Indeed, conformational changes that occur upon protein adsorption are difficult to assess with quantitative methods because of the low amount of adsorbed amounts. Garcia et al.[103] showed that FN conformational changes can be detected by different antibodies recognizing specific protein motifs. Antibody binding also depends on the state of the molecule (stretched versus relaxed).[104] Such FN conformational changes can control switching between proliferation and differentiation. Last but not least, other proteins present in a much lower amount in the serum may act in synergy with the preadsorbed proteins.

Gold nanoparticles (AuNPs) were used as a tool for depositing cell adhesion proteins, fibronectin, and ephrinB3 on top of PEM films.[105] The authors studied cancer cell adhesion and found that it was affected by nanoparticle density, an optimum being observed for an intermediate nanoparticle density. Drastic changes in cell adhesion were observed, with the formation of protractions (lamellipodia and filopodia). Of note, the influence of the nanotopology here was higher than the influence of the coating of the AuNP. Interestingly, the authors also studied ephrin signaling by quantifying the expression of paxillin. They found that it was more effective when ephrin B3 was presented from the AuNP than when it was directly attached to the polymer film.

4.1.2. Grafting ECM-Derived Peptides. Selectivity, that is, specificity in adhesion, can be achieved by grafting peptides (Figure 4B) that are known to interact with specific cell adhesion receptors, typically integrins. In this case, only short sequences of ECM proteins are considered. The most prominent example is that of the RGD sequence, RGD being an important integrin-binding motif region in FN and COL, which has already been grafted to polymers using various strategies.[8] Using PEM, two different strategies have been developed. The first consists of grafting the peptide to one of the polyelectrolytes and then adsorbing the modified polyelectrolyte as a regular layer. The synthetic step is thus performed aside from the film buildup. PEM films with poor adhesion are excellent candidates for such functionalization, which was applied using PAH-RGD and PGA-RGD for cell attachment.[66,106] PGA-RGD added as the outermost layer was shown to have a beneficial influence on osteoblast adhesion and proliferation.[66] In an elegant work by Werner et al.,[107] it was shown that a laminin5-derived peptide grafted to PGA could induce specific cell adhesive structures in epithelial cells called hemidesmosomes and specifically activate β4 integrins. PAH-RGD was also found to increase adhesion on (PAH/PAA) films.[108] The osteoblasts exhibited a better differentiated phenotype on the pH 2.0 films than on the pH 6.5 films, with respect to calcium deposition. However, incorporation of another peptide (LHRRVKI) known to be a heparin binding domain did not support cell adhesion, growth, or matrix mineral deposition.

The second strategy consists of directly coupling the RGD peptide onto the film, using the carbodiimide EDC as a coupling agent.[109] This was achieved on (HA/CHI) films

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deposited on titanium. Osteoblast cells adhered and proliferated much better in the presence of the grafted peptide.

Importantly, however, the question has been raised as to whether these chemical modifications in the polyelectrolytes may alter other physical chemical properties, such as protein adsorption or mechanical properties, in turn influencing cell adhesion and proliferation. These points were investigated by Thompson et al.[110] and Schneider et al.[111] who measured the mechanical properties of the films with or without modified polyelectrolytes.

4.2. PEM Films Modified with Growth Factors and Hormones. Another way to render the films bioactive and induce specific cell responses is to use bioactive molecules, such as growth factors and hormones, that control cell proliferation and differentiation.

4.2.1. Grafting of Hormones. A short peptide hormone, α-MSH (alpha-melanocyte-stimulating hormone), with anti-inflammatory properties, has been successfully integrated into multilayer films. Initially coupled with PLL, α-MSH was effective toward melanoma cells that were induced to produce melanocortin.[112] Then, coupled with PGA and introduced into (PLL/PGA) films, it was efficient in annihilating the effect of a bacterial endotoxin that stimulated an inflammatory response in human monocytic cells.[113] The morphology of the monocytes was also affected by α-MSH as the cells formed many "fiberlike" protrusions not visible on standard (PLL/PGA) films.

4.2.2. Presenting Matrix-Bound Growth Factors. It should be noted that grafting proteins is more difficult to control than grafting peptides, as the protein should not be denatured during the reaction. Conversely, as the precise active site in a protein is not always known or requires a special 3D conformation, the presence of the full length protein (and not of a peptide sequence) can be required to achieve bioactivity.

Up to now, two major strategies have been employed to provide films with a specific bioactivity using larger molecules such as growth factors (Figure 4A): (i) adsorption of the bioactive molecule as a regular layer. The deposition step has to be carefully chosen depending on the physicochemical properties of the protein (isoelectrice point, solubility depending on solution pH, and ionic strength) and (ii) adsorption and possibly postloading the bioactive molecule in the as-prepared films. Depending on the film's internal structure (thickness, porosity, internal groups, and charges), the bioactive molecule may simply adsorb at the film's surface or diffuse in it. Of note, if the PEM film is made of biodegradable polymers, then the bioactive molecule will be delivered in solution (Figure 4C). If it is not biodegradable, the bioactive molecule may partially diffuse out of the PEM film and is delivered to the basal surface of the cell.

Growth factors (i.e., proteins that can control growth and maturation of tissues, cell proliferation, division, and differentiation) are especially interesting in this respect.

The first strategy was applied for basic fibroblast growth factor (bFGF or FGF-2), a factor that is involved in cell proliferation and differentiation of a wide variety of cells and tissues. This factor was also deposited with CS by Shen et al.,[114] who built (FGF-2/CS) films. These authors showed that approximately 30% of the incorporated FGF-2 was released within 8 days. *In vitro* cell culture found that the fibroblasts showed star-like morphology with plenty of pseudopods on the FGF-2 incorporated collagen film after 1 day of culture, and the collagen films assembled with FGF-2 had better bioactivity than that of the virgin one and the FGF-2 control.

The second strategy of adsorption on top of PEM films was also applied to FGF-2 by Tezcaner et al.[43] Using (PLL/CSA) films with adsorbed FGF-2, these authors showed that FGF-2 increased the number of photoreceptor cells attached and maintained the differentiation of rod and cone cells.

(CHI/HEP) film construction was achieved in the presence and absence of adsorbed FN and FGF-2.[115] The functional response of bone marrow-derived ovine MSCs to these PEM coatings deposited on TCPS and titanium was investigated. These authors found that FGF-2 adsorbed to heparin-terminated PEMs with adsorbed FN induced greater cell density and a higher proliferation rate of MSCs than any of the other conditions tested, including delivery of the FGF-2 in solution, at an optimally mitogenic dose. This effect was observed for PEM-coated TCPS. However, surprisingly, the same effects were not observed when the FGF-2 was delivered from PEM adsorbed on titanium, and the response of ovine MSCs to adsorbed FGF-2 was not as strong as the response to FGF-2 delivered in solution. This requires further investigation.

More recently, the loading and release of FGF-2 from synthetic hydrolytically degradable multilayer thin films of various architectures were explored by Hammond's group.[116] Three parameters were studied: number of layers, counter-polyanion (heparin or chondrotin sulfate), and type of degradable polycation. The incorporated amounts were found in the range 7−45 ng/cm^2 of FGF-2, and the release time varied between 24 h and approximately 5 days. The effective bioactivity of the released FGF-2 was proved *in vitro*, as it promoted the proliferation of MC3T3-E1 preosteoblast cells. Interestingly, FGF-2 released from LbL films demonstrated increased ability of up to 8 times the negative control values to enhance proliferation, compared to the free FGF-2 (about 2 times). Importantly, none of the other film components (including CS and HEP) showed any proliferative effect on the preosteoblasts.

FGF-2 adsorption via heparin-based PEM was also applied to decellularized porcine aortic heart valve leaflets,[117] which are used in the replacement of diseased aortic valves. FGF-2 was found to be released slowly from the valve and was sustained over 4 days, while its biological activity was preserved, as proved by increased fibroblast viability.

As the immobilized growth factors can maximally retain their bioactivity, the LBL assembly would be a potential approach for constructing a bioactive substrate for biomedical applications.

Additional bioactivity can be provided for (PSS/PAH) films by adsorbing growth factors onto them or by adding them before the last deposited layer. Here again, the charge of the polyelectrolyte can influence cell adhesion, metabolic activity, and adsorbed growth factor. The first proof of effective bioactivity of two nerve growth factors was provided by Vodouhe et al.[118] First, the authors showed that better neuron viability was observed on (PSS/PAH) and (PLL/PGA) films in comparison to a simple monolayer. Second, they embedded two nerve growth factors, brain-derived neurotrophic factor (BDNF) and semaphoring 3A (Sema3A) into (PSS/PAH) films before depositing a final PSS layer. The adsorbed protein amounts were 95 ng/cm^2 for BDNF and 25 ng/cm^2 for Sema3A. They evidenced that the embedded proteins remained functional and available, even under two layers of polyelectrolytes. Both proteins modified the growth of the neurons either by increasing it (BDNF) or by reducing neurite length (Sema3A). Such PEM films would allow the direct presentation

of growth factors in the injury environment for promoting repair of neuronal tissue.

Vautier et al.[119] modified the surface of porous titanium implants with polyelectrolyte multilayer (PEM) films functionalized with vascular endothelial growth factor (VEGF). Of the two PEM systems investigated, poly(L-lysine)/poly(L-glutamic acid) (PLL/PGA) and (PAH/PSS), they selected a (PAH/PSS) film made of four layer pairs ending with PSS for both its high efficiency to adsorb VEGF and its biocompatibility toward endothelial cells. Furthermore, they showed that it stimulated the proliferation of endothelial cells.

They demonstrated that VEGF adsorbed on $(PAH/PSS)_4$ maintains its bioactivity *in vitro* by measuring the phosphorylation of the endothelial VEGF receptor VEGFR2 and the specific activation of the mitogen-activated protein kinases (MAPK) ERK $^1/_2$ pathway. This effect was correlated with specific activation of intracellular signaling pathways induced by successive phosphorylation of the endothelial VEGF receptor VEGFR2 and mitogen-activated protein kinases (MAPK) ERK$^1/_2$.

Cells capable of differentiating and, in particular, stem cells that are multi- or pluripotent cells, are currently the subject of several studies thanks to their potential applications in tissue repair *in situ* and tissue engineering. Here again, growth factors are of special interest.

Dierich et al.[120] were the first to show the use of PEM films for the differentiation of embryonic bodies (EBs) into cartilage and bone. A poly(L-lysine succinylated)/PGA film, into which BMP-2 (bone morphogenetic 2) and TGFβ1 (transforming growth factor 1) had been embedded, was chosen for this purpose. They found that both BMP-2 and TGFβ1 needed to be present simultaneously in the film to trigger proteoglycan production and to drive the EBs to cartilage and bone formation. The same authors subsequently investigated the effect of a growth factor, BMP-4, and its antagonist, Noggin, embedded in a PLL/PGA film on tooth development.[121] They showed that these films can induce or inhibit cell death in tooth development and that the biological effects of the active molecules are conserved. The functionalized PEMs could thus act as efficient delivery tools for activating cells. This approach shows promise, as it could be used to finely reproduce architectures with cell inclusions and to provide tissue organization.

BMP-2 is another member of the BMP family that is particularly interesting for accelerating bone healing.[122] BMP-2 has been inserted into a film as a regular layer, but the successive washing steps do not allow a high amount of BMP-2 to be retained (less than 100 ng per substrate).[123] When it is combined with hydrolytically degradable polycations (β-amino-esters), several μg can be loaded and 10 μg of BMP-2 are released over a period of two weeks *in vitro*.[124] Of note, there was no initial burst (less than 1% is released in the first 3 h), as compared with commercial collagen matrices, which can release up to 60% of BMP-2. BMP-2 released from LbL films retains its ability to induce bone differentiation in MC3T3 preosteoblasts, as measured by induction of alkaline phosphatase and stains for calcium. *In vivo*, BMP-2 film coated polymeric scaffolds implanted intramuscularly in rats were shown to induce bone formation.

The adsorption strategy was also applied to BMP-2, which was shown to diffuse in cross-linked (PLL/HA) films.[16] Indeed, (PLL/HA) films are a reservoir for BMP-2, as very high amounts can be obtained (up to 7 $\mu g/cm^2$), and only a small fraction was released initially. The amount of BMP-2 trapped could be adjusted by varying both the number of layers in the film and the initial BMP-2 concentration in solution. The effective proof of bioactivity was obtained on myoblast cells: cells differentiated into myotubes on cross-linked (PLL/HA) films without BMP-2 in the films, but they differentiated into osteoblasts in a dose-dependent manner when cultured on the BMP-2 loaded films. The expression of alkaline phosphatase, a marker for osteoblastic activity, was dependent on the amount of BMP-2 loaded into the films.[16]

If a mixture of heparin and hyaluronan is used as a polyanion in the film buildup, heparin is found to be preferentially incorporated. In this case, thinner and denser films are obtained, onto which only a small amount of BMP-2 can be adsorbed. Interestingly, the alkaline phosphatase (ALP) production by myoblast cells was found to be solely correlated to the amount of BMP-2 adsorbed or trapped in the film, independent of the film's internal chemistry.[125] Furthermore, the bioactivity of BMP-2 loaded in cross-linked (PLL/HA) films deposited on TCP/HAP granules, biomaterials used in orthopedic surgery as a bone substitute, was confirmed by *in vivo* studies in rats.[126] Induction of bone around the PEM-film coated implant (i.e., osteo-induction) was proved to be due to the sole presence of BMP-2, with the film itself being inert. In addition, the PEM film did not induce an inflammatory response in the surrounding tissues.

4.3. Other Types of Specific Interactions. A receptor that is especially important for the healing of the endothelium is CD34. In the cardiovascular field, stent implantation is a common procedure, which may subsequently lead to in-stent restenosis (i.e., obstruction of the vessel) or even stent thrombosis. It is therefore important to stimulate healing of the endothelium in appropriate conditions. Ji et al. developed a strategy to mimic the natural endothelium healing mechanism that consists in stimulating neighboring endothelial cell (EC) migration or capturing the circulating endothelial cells directly from the blood circulation.[77] To this end, they immobilized an anti-CD34 antibody on heparin/collagen multilayers. They found that the PEM coating with or without the anti-CD34 antibody functionalization preserved good hemocompatibility but also promoted cell attachment and growth, notably, in a nonselective manner. However, the anti-CD34 antibody functionalized heparin/collagen multilayers could specifically promote the attachment and growth of vascular ECs at the expense of smooth muscle cells.

Specificity in the interaction may also be observed for other types of receptor. HA, which is an important polysaccharide component of ECM, is known to interact with several receptors. Among them is CD44, a cell surface glycoprotein involved in cell/cell adhesion, cell adhesion, and migration. B lymphocyte adhesion onto (CHI/HA) films was investigated by varying the deposition conditions, especially ionic strength and pH.[127] The authors showed that there was a specific interaction between the CD44 receptor in lymphocyte cells and HA. Furthermore, the deposition conditions of the films had an influence on the interaction, low pH and added salt being the preferred conditions for higher cell binding. This interaction was increased in conditions that favor loops and tails in HA. However, they also noticed that CHI-terminated films prepared without NaCl in the deposition solutions presented a similar high lymphocyte binding efficiency, which they attributed to increased electrostatic contributions.

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The same group showed that it is possible to attach a superparamagnetic PEM patch to the membranes of T- and B-lymphocytes using CD44-HA interactions.[128] B-cells responded to an applied magnetic field, and T-cells continued to chemokinetically migrate on intercellular adhesion molecule (ICAM)-coated surfaces following patch attachment.

However, it should be noted that, for other films that contain HA, such as (PLL/HA) films, no specific interaction with HA receptors has been evidenced.[129] This might be due to the fact that the films are cross-linked, which may affect the presentation of HA to the cell receptors as a result of its entanglement with PLL chains.

5. TOWARD MULTIFUNCTIONALITY

PEM coatings may offer new tools to tissue engineers and biophysicists, who need well controlled and well characterized biomimetic matrices. PEM coatings offer new potentialities when compared to classic synthetic materials, such as polyacrylamide gels[9] or poly(ethylene glycol),[130] by making use of both covalent and noncovalent interactions. The potentialities for manufacturing multifunctional coatings that combine, for instance, spatial organization and bioactivity, adjustable stiffness and chemistry, or adjustable stiffness and bioactivity, are apparently unlimited. Recent examples illustrate that we are now entering an area of new developments for the design of multifunctional films.

5.1. Three-Dimensional Microenvironments Containing Bioactive Films. PEM films can be considered as 2D matrices, even if they can be several tens of micrometers thick, in the sense that they cannot provide a sufficiently porous 3D scaffold for the cells to grow in. However, PEM films can be deposited on porous materials and provide additional properties for the biomaterial surface.

In a study describing further efforts to provide stem cells with a biomimetic niche environment, Nichols et al.[131] built an elegant scaffold with an inverted colloidal crystal topography reminiscent of bone marrow architecture, which was further coated with albumin/PDDA films. Bone marrow stromal cells were first allowed to attach to the scaffold. Subsequently, CD34+ hematopoietic stem cells were seeded in the scaffold to create a three-dimensional coculture. The authors demonstrated that the scaffold supports CD34+ cell expansion and B lymphocyte differentiation with production of antigen specific IgG antibodies. Recently, the same group achieved a further step toward mimicking the cell microenvironment of the bone marrow and thymus by presenting a Notch ligand (delta-like 1, DL-1) at the surface of the PEM film.[132] For this purpose, they used mononuclear cells derived from human umbilical cord that were positive for the surface marker CD34 (CD34+). After 28 days of growth on the PEM-coated colloidal scaffolds, the cells were found to be CD4+ and CD8-, an observation that was specifically due to the presence of the DL-1 Notch ligand. Without the DL-1 coating, the cells were shown to express a CD34 for 2 weeks, which indicated that the PEM-coated scaffold stimulated *ex vivo* hematopoietic stem cell expansion without notch signaling. In addition, the cells progressively developed their own ECM.

3D microwells are increasingly used for cell culture arrays.[133] Lynn et al. developed an approach to the fabrication and selective functionalization of amine-reactive polymer multilayers on the surface of 3D-polyurethane-based microwells.[134] These authors prepared film-coated arrays that could be chemically functionalized postfabrication by treatment with different amine-functionalized macromolecules or small molecule primary amines. They showed that spatial control over glucamine functionalization yielded 3D substrates that could be used to confine cell attachment and growth to microwells for periods up to 28 days. A dual functionalization could also be achieved by sequential treatment with two different fluorescently labeled cationic polymers: functionalization of the surface of the wells with one polymer and the regions between the wells with a second. This approach to dual functionalization opens perspectives for the long-term culture and maintenance of cell types, such as stem cells.

5.2. Bioactivity of Two Different Growth Factors. The group of Professor Hammond showed that is it possible to release, at precise doses, two types of potent growth factors, osteogenic BMP-2 (to induce bone regeneration) and angiogenic VEGF165 (to induce neovascularization), in different ratios in a degradable [poly(b-amino ester)/polyanion/growth factor/polyanion] LbL tetralayer repeat architecture.[135] The amount of biologically active molecules loaded was precisely controlled by varying the number of tetralayers. Very interestingly, both growth factors were shown to retain their bioactivity *in vitro*: BMP-2 initiated differentiation of preosteoblastic cells and VEGF induced proliferation of endothelial cells. The authors also showed that the mineral density of the ectopic bone formed was about 33% higher in the case of the dual release (Figure 5), as compared to BMP-2 alone, which they attributed to an increased local vascular network.

5.3. Mechanical Stimulation and Delivery of Bioactive Molecules. PEM film properties may also be combined with mechanical stimulation or with electrochemical stimulation. In an elegant study, Lavalle et al. showed that (PLL/HA) films coated with PDMS substrates can be stretched and release an enzyme that is loaded in the bulk of the film and capped with a synthetic PEM barrier.[136] The biocatalytic activity of the film could be switched on/off reversibly by mechanical stretching, which exposed enzymes through the capping barrier, similar to the mechanisms involved in proteins during mechanotransduction. This opens new possibilities for triggering the release of bioactive molecules from the film "bulk".

Interestingly, a study by Schaaf et al.[137] showed that the adhesive state of fibroblasts can be changed from cytophobic to cytophilic simply by stretching a film with a cell repellent phosphatidylcholine−PAA layer as the final layer. In the aforementioned examples, the substrate supporting the PEM film is stretched in a controlled fashion.

The ability to construct stable ECM-based films on PDMS[138] has particular relevance in mechanobiology, microfluidics, and other applications. A combination of PEM with microfluidics appears to be highly promising. It was shown that PEM films can be deposited in a microfluidic device and that a pH gradient could be generated during multilayer formation.[139] The authors showed that cells started to migrate from the films built at pH 5 to those built at pH 9. Developments of PEM films in combination with electrochemistry also appear promising because biomolecules can be released from the films[140] as well as whole cell sheets.[141]

Mechanical stimulus can also be provided by varying the film's stiffness. As mentioned previously, cells can respond actively to the rigidity of a substrate by exerting forces on it, which allows them to adhere and spread more or less, depending on the rigidity of the underlying matrix. Using (PLL/HA) films, we have shown that it is possible to combine

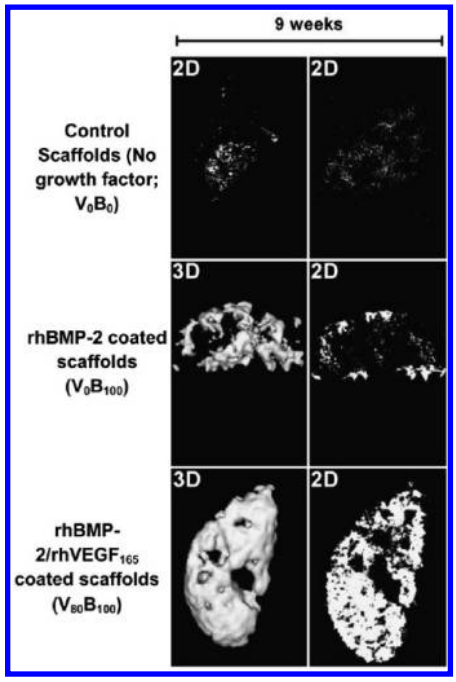

Figure 5. Two dimensional microCT scans (2D) and matched three-dimensional reconstructions (3D) of excised PCL-bTCP half disk scaffolds, which were implanted in the intramuscular region of rats. Implants were coated with (i) no growth factor, (ii) 6 μg of single growth factor rhBMP-2, and (iii) 6 μg of single growth factor rhBMP-2 followed by 4 μg of rhVEGF165. The amount of growth factor loaded was determined by fabricating triplicate companion copies along with the implanted scaffolds, releasing the growth factors *in vitro* and performing ELISA detection assays. (Top row) Control scaffolds without growth factors produce no detectable bone over the duration of the study. Low levels of backscatter is caused by the polymer. (Middle row) In single growth factor rhBMP-2 films lacking rhVEGF165, bone formation is restricted to the periphery of the scaffold at 4 weeks (images not shown) and 9 weeks. (Bottom row) As a result of increased vascularity, scaffolds releasing rhVEGF165 demonstrate a smooth, continuous profile in the ectopically formed bone which matures from 4 weeks to 9 weeks to fill the entire scaffold. In all the images, the bone formed takes the shape of the scaffold and grows inward when VEGF is present. Images are an isosurface rendering at 0.25 surface quality factor at a level threshold of 640, as defined by the proprietary Microview software from GE Healthcare (reproduced with permission from ref 116, copyright 2011 Elsevier).

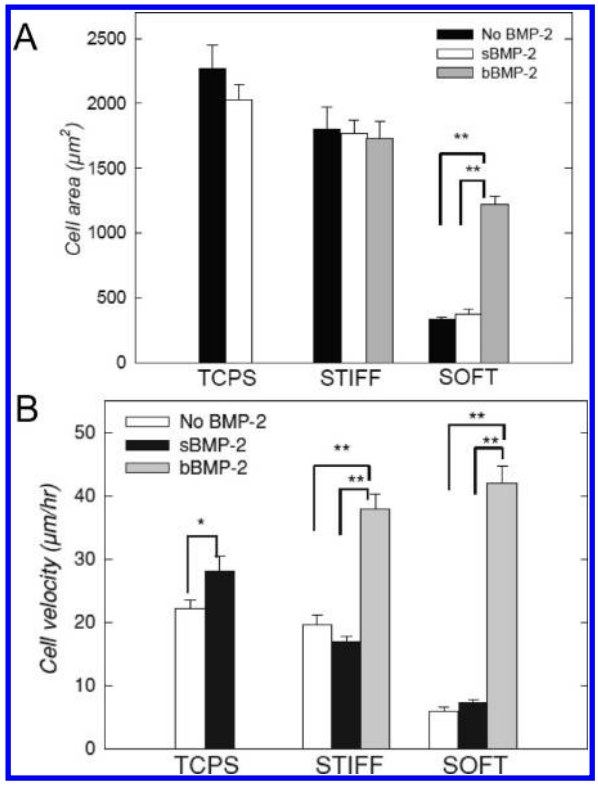

Figure 6. Sustained effect of matrix-bound BMP-2 on cell morphology and on cell migration. Cell morphology is observed 16 h after plating the cells. Actin and nucleus staining of C2C12 cells revealed a well spread morphology on the glass control substrate as well as on stiff (PLL/HA) films in the presence or in the absence of BMP-2, independently of the presentation mode of BMP-2 ("soluble" versus "matrix-bound"). Conversely, for soft (PLL/HA) films, sBMP-2 did not induce any noticeable effect on cell spreading but bBMP-2 induced a striking increase in cell spreading (images not shown). (a) Cell surface area is plotted for the different conditions. This shows the drastic increase in cell spreading in response to bBMP-2 on soft films is sustained after initial adhesion. (b) Migration velocity (μm/hour) of C2C12 cells cultured on different substrates: standard tissue culture polystyrene (TCPS) and either stiff or soft films. Matrix-bound BMP-2 greatly increases cell velocity on stiff films and even more on soft films with bBMP-2. The soluble form of BMP-2 had no significant effect compared to the condition where no BMP-2 is added, except on TCPS. $^{**}p < 0.005$. (Reproduced with permission from ref 142, copyright Wiley 2011.)

film stiffness and presentation of the BMP-2 growth factor by the matrix.[142] Here, the PEM film offers the possibility of providing two independent stimuli: a mechanical stimulus and a biochemical stimulus that is known to impact cell differentiation.[16] By preparing films of different stiffness and by loading a known amount of BMP-2, we revealed that BMP-2 has a drastic effect on early cell adhesion (Figure 6a), as well as on cell migration (Figure 6b). This effect was especially potentiated when BMP-2 was presented from soft films. First, this highlights that biochemical stimuli can override mechanical stimuli in certain conditions. Second, this also proves that BMP-2 has an effect not only on cell differentiation but also possibly on the early stages of cell adhesion. This opens a route for studies on the interplay between growth factor presentation from the matrix (and associated cell signaling) and cell adhesion receptors involved in rigidity sensing.

Other types of stimulation such as light-triggered release of activated molecules[143] might also be used in the future to locally delivery bioactive molecules to cells, as they have shown promise in the delivery of cargo from microcapsules adsorbed onto the films.[144]

6. EVALUATION OF TOXICITY AND *IN VIVO* STUDIES

For translation of PEM-coated surfaces or PEM microcapsules into medicine, both the efficiency and toxicity of the PEM assemblies must be evaluated. It is already known that several single polycations are potentially cytotoxic, depending on the dose and site of injection. This is valid for PEI and PLL,[145] and for CHI as well.[146] Systematic studies for each specific case are thus required.

The biocompatibility of a single PEI layer was tested on both fibroblastic and osteoblastic cells. Pure titanium (Ti) and nickel−titanium (NiTi) alloy were coated with PEI, and morphology, adhesion, and viability were assessed for up to 7 days after seeding. The results show that the cells were less viable and proliferated less on PEI-coated titanium than on the

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control, suggesting that PEI is potentially cytotoxic.[147] On the other hand, (PSS/PAH) films deposited on human umbilical arteries showed good grafting behavior and no inflammation in a rabbit model after 12 weeks of implantation.[148] Coronary stents have also been coated with (CHI/HEP) films, and they were tested *in vitro* and *in vivo* in a pig model.[149] This PEM coating was found to be safe and efficient in promoting re-endothelialization and intimal healing after stent implantation, in addition to having good hemocompatibility. Similarly, (PLL/PGA) films have been coated on a tracheal prosthesis and implanted for up to three months.[150] For prostheses modified by PGA ending multilayer films, a more regular and less obstructive cell layer was observed on the endoluminal side, compared to those modified by PLL ending films. An anti-inflammatory peptide grafted to PGA was found to be bioactive *in vivo*. No inflammation was observed in the case of BMP-2 delivered intramuscularly.[124,126]

De Geest et al. carried out an interesting study using a terrestrial slug, namely, *Arion lusitanicus*, as a nonvertebrate model organism to investigate mucosal irritation.[151] This slug has been used to test several pharmaceutical, as well as health care components *in vivo*[152] as an alternative to tests in mice, rabbits, or other nonhuman animals. They investigated the mucosal irritation potency of several classes of biopolymers, synthetic polyelectrolytes, and the reactive polyelectrolytes of oppositely charged polyelectrolytes, their complexes, and hollow multilayer capsules, which they intend to use in vaccines.[153] They found that single polyelectrolyte components induced tissue irritation. However, very interestingly, this response was dramatically reduced upon complexation with an oppositely charged polyelectrolyte, regardless of whether the polyelectrolytes were randomly complexed in water or assembled in a controlled fashion in multilayer capsules.

The chemical modification of polycations is also possible for decreasing their potential toxicity. By using PLL modified with PEG groups (PLL-g-PEG) and by assembling them with alginate, Chaikof et al. showed that individual pancreatic islets can be coated with (PLL-g-PEG/ALG) multilayer films.[154] These authors also demonstrated that additional biological specificity can be provided for the islets by depositing specific groups (such as biotin or azide functionalized-PEG). Very interestingly, they also showed that the functional capacity of islets to release insulin in a glucose-responsive manner was not adversely influenced by the PEM film. Indeed, the islets engineered with PEMs secreted statistically similar amounts of insulin at both basal and high glucose concentrations compared to untreated controls. Furthermore, by implanting these islets *in vivo* into mice through the portal vein and into the liver microvasculature, they proved that the survival and function of these PEM-coated cells.

All together, these studies show that it is possible to design PEM films with bioactive properties *in vivo*, which can be fully integrated *in vivo* without any noticeable toxicity. Here again, each engineered PEM system will have to be studied in the framework of a specific application.

8. CONCLUDING REMARKS

In the last 5 years, there have been considerable developments in synthetic and natural PEM assemblies for the coating of biomaterial surfaces and tissue engineering. An important aspect is the dynamic nature of mono- or multicellular systems (interactions between cells or cell/matrix) that occur over several hours, days, and weeks. Based on this survey, it appears that better defined applications and multifunctionalization using several strategies simultaneously have emerged. The various strategies that are used to noncovalently localize bioactive adhesion molecules and growth factors appear highly promising for future *in vivo* studies on tissue regeneration as well as for more fundamental mechanistic studies. PEMs may serve as new biomimetic matrices with controlled physical properties and controlled presentation of biochemical moieties, for investigating cell/material or cell/cell interactions. It is now acknowledged that the means of presentation of a bioactive molecule is a key point in its bioactivity and that matrix-bound presentation is much more physiologic than delivering growth factors in solution.[155] As we have seen here that many growth factors retain their bioactivities inside or on top of PEM films, we foresee that PEMs will help answer important biological questions such as the following: How do matrix-bound molecules interact with cell receptors and transduce biochemical signals, as compared to soluble molecules added in the culture medium? It will also be interesting to unravel the structure of the bioactive molecules inside PEM films and to understand the molecular mechanisms at the basis of their preserved bioactivity. General rules may emerge. This will require the use of new biochemical and biophysical analytical tools. PEMs will undoubtedly find a place alongside other well established materials such as polyacrylamide or polyethylene glycol hydrogels, which require covalent grafting for the coupling of chemical ligands.

The potentialities for manufacturing multifunctional PEM coatings are apparently unlimited. The design and pertinence of such architectures will rely on a strong multidisciplinary approach and will require collaboration between engineers, physical chemists, organic chemists, biochemists, and cell and stem cell biologists. In the burgeoning field of stem cells, PEM films also appear to offer a tool to maintain stemness or to guide cell differentiation. Besides being a 2D coating, their application in 3D mimetic architectures will be an original means of controlling supra-cellular organization. Thus, the reciprocal interactions between active cells and active PEM surfaces offer tremendous potentialities that will be explored in the future.

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ABBREVIATIONS

ALG = alginate.
AuNP = gold nanoparticles.
BDNF = brain derived neurotrophic factor.
BMP = bone morphogenetic proteins.
CHI = chitosan.
CS = chondroitin sulfate and CSA for chondroitin sulfate A.
CLSM = confocal laser scanning microscopy.

COL = collagen.
EDC = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.
EPC = endothelial progenitor cell.
ESC = embryonic stem cells.
FGF-2 (or bFGF) = basic fibroblast growth factor.
FN = fibronectin.
ICM = inner cellular mass.
HA = hyaluronan.
HEP = heparin.
LbL = layer-by-layer.
MAPK = mitogen-activated protein kinases.
MSC = mesenchymal stem cells.
α-MSH = alpha-melanocyte-stimulating hormones.
NGF = nerve growth factor.
PA = polyacrylamide.
PDMS = poly(dimethylsiloxane).
PEI = poly(ethylene)imine.
PLL = poly(L-lysine).
PAH = poly(allylamine) hydrochloride.
PEM = polyelectrolyte multilayer.
PSS = poly(styrene) sulfonate.
SMC = smooth muscle cells.
SWNT = single-walled carbon nanotube.
TCPS = tissue culture polystyrene.
VB = vinylbenzyl.
VEGF = vascular endothelial growth factor.

REFERENCES

(1) Decher, G.; Hong, J. D.; Schmitt, J. *Thin Solid Films* **1992**, *210*−*211*, 831−835. Lvov, Y.; Decher, G.; Haas, H.; Mohwald, H.; Kalachev, A. *Phys. B* **1994**, *198*, 89−91.
(2) Castner, D. G.; Ratner, B. D. *Surf. Sci.* **2002**, *500*, 28−60.
(3) Hubbell, J. A. *Curr. Opin. Biotechnol.* **1999**, *10*, 123−129.
(4) Fattori, R.; Piva, T. *Lancet* **2003**, *361*, 247−9.
(5) Langer, R.; Vacanti, J. P. *Science* **1993**, *260*, 920−926.
(6) Drury, J. L.; Mooney, D. J. *Biomaterials* **2003**, *24*, 4337−51.
(7) Chen, C. S.; Mrksich, M.; Huang, S.; Whitesides, G. M.; Ingber, D. E. *Science* **1997**, *276*, 1425−1428.
(8) Hersel, U.; Dahmen, C.; Kessler, H. *Biomaterials* **2003**, *24*, 4385−4415.
(9) Wang, Y. L.; Pelham, R. J. Jr. *Methods Enzymol.* **1998**, *298*, 489−496.
(10) Engler, A. J.; Sen, S.; Sweeney, H. L.; Discher, D. E. *Cell* **2006**, *126*, 677−89.
(11) Hudalla, G. A.; Murphy, W. L. *Adv. Funct. Mater.* **2011**, *21*, 1754−1768.
(12) Tang, Z.; Wang, Y. l.; Podsiadlo, P.; Kotov, N. A. *Adv. Mater.* **2006**, *18*, 3203−3224.
(13) Shiratori, S. S.; Rubner, M. F. *Macromolecules* **2000**, *33*, 4213−4219.
(14) von Klitzing, R. *Phys. Chem. Chem. Phys.* **2006**, *8*, 5012−33.
(15) Garza, J. M.; Jessel, N.; Ladam, G.; Dupray, V.; Muller, S.; Stoltz, J. F.; Schaaf, P.; Voegel, J. C.; Lavalle, P. *Langmuir* **2005**, *21*, 12372−12377.
(16) Crouzier, T.; Ren, K.; Nicolas, C.; Roy, C.; Picart, C. *Small* **2009**, *5*, 598−608.
(17) Kwok, C. S.; Mourad, P. D.; Crum, L. A.; Ratner, B. D. *Biomacromolecules* **2000**, *1*, 139−148.
(18) Boudou, T.; Crouzier, T.; Ren, K.; Blin, G.; Picart, C. *Adv. Mater.* **2010**, *22*, 441−67.
(19) Schönhoff, M.; Ball, V.; Bausch, A. R.; Dejugnat, C.; Delorme, N.; Glinel, K.; von Klitzing, R.; Steitz, R. *Colloids Surf., A* **2007**, *303*, 14−29. Jaber, J. A.; Schlenoff, J. B. *Curr. Opin. Colloid Interface Sci.* **2006**, *11*, 324−329. Sukhishvili, S. A.; Kharlampieva, E.; Izumrudov, V. *Macromolecules* **2006**, *39*, 8873−8881.
(20) Hammond, P. T. *Adv. Mater.* **2004**, *16*, 1271−1293.
(21) Lynn, D. M. *Adv. Mater.* **2007**, *19*, 4118−4130.
(22) Zhang, L.; Zhao, W. H.; Rudra, J. S.; Haynie, D. T. *ACS Nano* **2007**, *1*, 476−486.
(23) Zelikin, A. N. *ACS Nano* **2010**, *4*, 2494−509.
(24) Pavlukhina, S.; Sukhishvili, S. *Adv. Drug Delivery Rev.* **2011**, *63*, 822−836.
(25) Jewell, C. M.; Lynn, D. M. *Adv. Drug Delivery Rev.* **2008**, *60*, 979−99.
(26) Lichter, J. A.; Van Vliet, K. J.; Rubner, M. F. *Macromolecules* **2009**, *42*, 8573−8586.
(27) Picart, C.; Gergely, C.; Arntz, Y.; Schaaf, P.; Voegel, J.-C.; Cuisinier, F. G.; Senger, B. *Biosens. Bioelectron.* **2004**, *20*, 553−561.
(28) Boura, C.; Menu, P.; Payan, E.; Picart, C.; Voegel, J.-C.; Muller, S.; Stoltz, J.-F. *Biomaterials* **2003**, *24*, 3521−3530.
(29) Mhamdi, L.; Picart, C.; Lagneau, C.; Othmane, A.; Grosgogeat, B.; Jaffrezic-Renault, N.; Ponsonnet, L. *Mater. Sci. Eng. C* **2006**, *26*, 273−281. Brunot, C.; Grosgogeat, B.; Picart, C.; Lagneau, C.; Jaffrezic-Renault, N.; Ponsonnet, L. *Dent. Mater.* **2008**, *24*, 1025−1022.
(30) Tryoen-Toth, P.; Vautier, D.; Haikel, Y.; Voegel, J.-C.; Schaaf, P.; Chluba, J.; Ogier, J. *J. Biomed. Mater. Res.* **2002**, *60*, 657−667.
(31) Wittmer, C. R.; Phelps, J. A.; Lepus, C. M.; Saltzman, W. M.; Harding, M. J.; Van Tassel, P. R. *Biomaterials* **2008**, *29*, 4082−4090.
(32) Kerdjoudj, H.; Boura, B.; Moby, V.; Montagne, K.; Schaaf, P.; Voegel, J.-C.; Stoltz, J.-F.; Menu, P. *Adv. Funct. Mater.* **2007**, 17.
(33) Kerdjoudj, H.; Berthelemy, N.; Rinckenbach, S.; Kearney-Schwartz, A.; Montagne, K.; Schaaf, P.; Lacolley, P.; Stoltz, J. F.; Voegel, J. C.; Menu, P. *J. Am. Coll. Cardiol.* **2008**, *52*, 1589−1597.
(34) Berthelemy, N.; Kerdjoudj, H.; Gaucher, C.; Schaaf, P.; Stolz, J. F.; Lacolley, P.; Voegel, J. C.; Menu, P. *Adv. Mater.* **2008**, *20*, 2674−2678.
(35) Guillaume-Gentil, O.; Semenov, O. V.; Zisch, A. H.; Zimmermann, R.; Voros, J.; Ehrbar, M. *Biomaterials* **2011**, *32*, 4376−4384.
(36) Mendelsohn, J. D.; Barrett, C. J.; Chan, V. V.; Pal, A. J.; Mayes, A. M.; F, R. M. *Langmuir* **2000**, *16*, 5017−5023.
(37) Hajicharalambous, C. S.; Lichter, J.; Hix, W. T.; Swierczewska, M.; Rubner, M. F.; Rajagopalan, P. *Biomaterials* **2009**, *30*, 4029−4036.
(38) Johansson, J. A.; Halthur, T.; Herranen, M.; Soderberg, L.; Elofsson, U.; Hilborn, J. *Biomacromolecules* **2005**, *6*, 1353−9.
(39) Zhang, J.; Senger, B.; Vautier, D.; Picart, C.; Schaaf, P.; Voegel, J.-C.; Lavalle, P. *Biomaterials* **2005**, *26*, 3353−3361.
(40) Ai, H.; Lvov, Y.; Mills, D.; Jennings, M.; Alexander, J.; Jones, S. *Cell Biochem. Biophys.* **2003**, *38*, 103−14.
(41) Cai, K.; Rechtenbach, A.; Hao, J.; Bossert, J.; Jandt, K. D. *Biomaterials* **2005**, *26*, 5960−71.
(42) Thierry, B.; Winnik, F. M.; Merhi, Y.; Silver, J.; Tabrizian, M. *Biomacromolecules* **2003**, *4*, 1564−1571. Picart, C.; Lavalle, P.; Hubert, P.; Cuisinier, F. J. G.; Decher, G.; Schaaf, P.; Voegel, J.-C. *Langmuir* **2001**, *17*, 7414−7424.
(43) Tezcaner, A.; Hicks, D.; Boulmedais, F.; Sahel, J.; Schaaf, P.; Voegel, J. C.; Lavalle, P. *Biomacromolecules* **2006**, *7*, 86−94.
(44) Crouzier, T.; Picart, C. *Biomacromolecules* **2009**, *10*, 433−42.
(45) Fu, J.; Ji, J.; Yuan, W.; Shen, J. *Biomaterials* **2005**, *26*, 6684−92. Boddohi, S.; Killingsworth, C. E.; Kipper, M. J. *Biomacromolecules* **2008**, *9*, 2021−2028.
(46) Servaty, R.; Schiller, J.; Binder, H.; Arnold, K. *Int. J. Biol. Macromol.* **2001**, *28*, 121−7.
(47) Cohen, M.; Klein, E.; Geiger, B.; Addadi, L. *Biophys. J.* **2003**, *85*, 1996−2005.
(48) Evanko, S. P.; Tammi, M. I.; Tammi, R. H.; Wight, T. N. *Adv. Drug Delivery Rev.* **2007**, *59*, 1351−65.
(49) Zhu, H.; Ji, J.; Tan, Q.; Barbosa, M. A.; Shen, J. *Biomacromolecules* **2003**, *4*, 378−386.
(50) Tan, Q. G.; Ji, J.; Zhao, F.; Fan, D. Z.; Sun, F. Y.; Shen, J. C. *J. Mater. Sci.* **2005**, *16*, 687−692.
(51) Chen, J. L.; Chen, C.; Chen, Z. Y.; Chen, J. Y.; Li, Q. L.; Huang, N. *J. Biomed. Mat. Res., Part A* **2010**, *95A*, 341−349.
(52) Grohmann, S.; Rothe, H.; Frant, M.; Liefeith, K. *Biomacromolecules* **2011**, *12*, 1987−1997.

(53) Mhanna, R. F.; Voros, J.; Zenobi-Wong, M. *Biomacromolecules* **2011**, *12*, 609−616.
(54) Salloum, D. S.; Schlenoff, J. B. *Biomacromolecules* **2004**, *5*, 1089−96.
(55) Ladam, G.; Schaaf, P.; Cuisinier, F. G. J.; Decher, G.; Voegel, J.-C. *Langmuir* **2001**, *17*, 878−882. Gergely, C.; Bahi, S.; Szalontai, B.; Flores, H.; Schaaf, P.; Voegel, J. C.; Cuisinier, F. J. *Langmuir* **2004**, *20*, 5575−82.
(56) Picart, C.; Ladam, G.; Senger, B.; Voegel, J.-C.; Schaaf, P.; Cuisinier, F. J. G.; Gergely, C. *J. Chem. Phys.* **2001**, *115*, 1086−1094.
(57) Ladam, G.; Gergely, C.; Senger, B.; Decher, G.; Voegel, J.-C.; Schaaf, P.; Cuisinier, F. J. G. *Biomacromolecules* **2000**, *1*, 674−687.
(58) Kidambi, S.; Lee, I.; Chan, C. *J. Am. Chem. Soc.* **2004**, *126*, 16286−16287.
(59) Ricotti, L.; Taccola, S.; Bernardeschi, I.; Pensabene, V.; Dario, P.; Menciassi, A. *Biomed. Mater.* **2011**, 6.
(60) Salloum, D. S.; Olenych, S. G.; Keller, T. C.; Schlenoff, J. B. *Biomacromolecules* **2005**, *6*, 161−7.
(61) Niepel, M. S.; Peschel, D.; Sisquella, X.; Planell, J. A.; Groth, T. *Biomaterials* **2009**, *30*, 4939−4947.
(62) Lavalle, P.; Gergely, C.; Cuisinier, F.; Decher, G.; Schaaf, P.; Voegel, J.-C.; Picart, C. *Macromolecules* **2002**, *35*, 4458−4465.
(63) Picart, C.; Mutterer, J.; Richert, L.; Luo, Y.; Prestwich, G. D.; Schaaf, P.; Voegel, J.-C.; Lavalle, P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 12531−12535.
(64) Richert, L.; Boulmedais, F.; Lavalle, P.; Mutterer, J.; Ferreux, E.; Decher, G.; Schaaf, P.; Voegel, J.-C.; Picart, C. *Biomacromolecules* **2004**, *5*, 284−294.
(65) Richert, L.; Lavalle, P.; Payan, E.; Stoltz, J.-F.; Shu, X. Z.; Prestwich, G. D.; Schaaf, P.; Voegel, J.-C.; Picart, C. *Langmuir* **2004**, *1*, 284−294.
(66) Picart, C.; Elkaim, R.; Richert, L.; Audoin, F.; Da Silva Cardoso, M.; Schaaf, P.; Voegel, J.-C.; Frisch, B. *Adv. Funct. Mater.* **2005**, *15*, 83−94.
(67) Mendelsohn, J. D.; Yang, S. Y.; Hiller, J.; Hochbaum, A. I.; Rubner, M. F. *Biomacromolecules* **2003**, *4*, 96−106.
(68) Olenych, S. G.; Moussallem, M. D.; Salloum, D. S.; Schlenoff, J. B.; Keller, T. C. *Biomacromolecules* **2005**, *6*, 3252−3258.
(69) Stevens, M. M.; George, J. H. *Science* **2005**, *310*, 1135−8.
(70) Ingber, D. *Curr. Opin. Cell Biol.* **1991**, *3*, 841−848.
(71) Engler, A.; Bacakova, L.; Newman, C.; Hategan, A.; Griffin, M.; Discher, D. E. *Biophys. J.* **2004**, *86*, 617−628.
(72) Lutolf, M. P.; Raeber, G. P.; Zisch, A. H.; Tirelli, N.; Hubbell, J. A. *Adv. Mater.* **2003**, *15*, 888−+. Moon, J. J.; Saik, J. E.; Poche, R. A.; Leslie-Barbick, J. E.; Lee, S. H.; Smith, A. A.; Dickinson, M. E.; West, J. L. *Biomaterials* **2010**, *31*, 3840−3847.
(73) Genes, N. G.; Rowley, J. A.; Mooney, D. J.; Bonassar, L. J. *Arch. Biochem. Biophys.* **2004**, *422*, 161−7.
(74) Young, J. L.; Engler, A. J. *Biomaterials* **2011**, *32*, 1002−9.
(75) Francius, G.; Hemmerle, J.; Ball, V.; Lavalle, P.; Picart, C.; Voegel, J.; Schaaf, P.; Senger, B. *J. Phys. Chem. C* **2007**, *111*, 8299−8306.
(76) Thompson, M. T.; Berg, M. C.; Tobias, I. S.; Rubner, M. F.; Van Vliet, K. J. *Biomaterials* **2005**, *26*, 6836−6845.
(77) Lin, Q. K.; Ding, X.; Qiu, F. Y.; Song, X. X.; Fu, G. S.; Ji, J. *Biomaterials* **2010**, *31*, 4017−4025.
(78) Olugebefola, S. C.; Ryu, S. W.; Nolte, A. J.; Rubner, M. F.; Mayes, A. M. *Langmuir* **2006**, *22*, 5958−62.
(79) Pozos Vazquez, C.; Boudou, T.; Dulong, V.; Nicolas, C.; Picart, C.; Glinel, K. *Langmuir* **2009**, *25*, 3556−3563.
(80) Jan, E.; Kotov, N. A. *Nano Lett.* **2007**, *7*, 1123−1128.
(81) Kocgozlu, L.; Lavalle, P.; Koenig, G.; Senger, B.; Haikel, Y.; Schaaf, P.; Voegel, J. C.; Tenenbaum, H.; Vautier, D. *J. Cell Sci.* **2010**, *123*, 29−39.
(82) Chen, A. A.; Khetani, S. R.; Lee, S.; Bhatia, S. N.; Van Vliet, K. J. *Biomaterials* **2009**, *30*, 1113−20.
(83) Schneider, A.; Francius, G.; Obeid, R.; Schwinté, P.; Frisch, B.; Schaaf, P.; Voegel, J.-C.; Senger, B.; Picart, C. *Langmuir* **2006**, *22*, 1193−1200.
(84) Richert, L.; Schneider, A.; Vautier, D.; Vodouhe, C.; Jessel, N.; Payan, E.; Schaaf, P.; Voegel, J. C.; Picart, C. *Cell Biochem. Biophys.* **2006**, *44*, 273−85.
(85) Richert, L.; Engler, A. J.; Discher, D. E.; Picart, C. *Biomacromolecules* **2004**, *5*, 1908−1916.
(86) Ren, K.; Crouzier, T.; Roy, C.; Picart, C. *Adv. Funct. Mater.* **2008**, *18*, 1378−1389.
(87) Brooke, G.; Cooka, M.; Blair, C.; Han, R.; Heazlewood, C.; Jones, B.; Kambouris, M.; Kollar, K.; McTaggart, S.; Pelekanos, R.; Rice, A.; Rossetti, T.; Atkinson, K. *Semin. Cell Dev. Biol.* **2007**, *18*, 846−858.
(88) Semenov, O. V.; Malek, A.; Bittermann, A. G.; Voros, J.; Zisch, A. *Tissue Eng., Part A* **2009**, *15*, 2977−2990.
(89) Niwa, H. *Development* **2007**, *134*, 635−46.
(90) Picart, C.; Senger, B.; Sengupta, K.; Dubreuil, F.; Fery, A. *Colloid Surf., A* **2007**, *303*, 30−36.
(91) Gheith, M. K.; Sinani, V. A.; Wicksted, J. P.; Matts, R. L.; Kotov, N. A. *Adv. Mater.* **2005**, *17*, 2663−2667.
(92) Jiang, C.; Tsukruk, V. *Adv. Mater.* **2006**, *18*, 829−840.
(93) Tang, Z.; Kotov, N. A.; Magonov, S.; Ozturk, B. *Nat. Mater.* **2003**, *2*, 413−418.
(94) Podsiadlo, P.; Tang, Z.; Shim, B. S.; Kotov, N. A. *Nano Lett.* **2007**, *7*, 1224−31.
(95) Srivastava, S.; Kotov, N. A. *Acc. Chem. Res.* **2008**, *41*, 1831−41.
(96) Skirtach, A. G.; Volodkin, D. V.; Mohwald, H. *ChemPhysChem* **2010**, *11*, 822−9.
(97) Kotov, N. A.; Winter, J. O.; Clements, I. P.; Jan, E.; Timko, B. P.; Campidelli, S.; Pathak, S.; Mazzatenta, A.; Lieber, C. M.; Prato, M.; Bellamkonda, R. V.; Silva, G. A.; Kam, N. W. S.; Patolsky, F.; Ballerini, L. *Adv. Mater.* **2009**, *21*, 3970−4004.
(98) Kam, N. W. S.; Jan, E.; Kotov, N. A. *Nano Lett.* **2009**, *9*, 273−278.
(99) Mehrotra, S.; Hunley, S. C.; Pawelec, K. M.; Zhang, L. X.; Lee, I.; Baek, S.; Chan, C. *Langmuir* **2010**, *26*, 12794−12802.
(100) Garcia, A. J.; Takagi, J.; Boettiger, D. *J. Biol. Chem.* **1998**, *273*, 34710−5. Friess, W. *Eur. J. Pharm. Biopharm.* **1998**, *45*, 113−136.
(101) Grinnell, F. *Trends Cell Biol.* **2000**, *10*, 362−365.
(102) Wittmer, C. R.; Phelps, J. A.; Saltzman, W. M.; Van Tassel, P. R. *Biomaterials* **2007**, *28*, 851−860.
(103) Keselowsky, B. G.; Collard, D. M.; Garcia, A. J. *J. Biomed. Mater. Res., Part A* **2003**, *66A*, 247−259.
(104) Little, W. C.; Schwartlander, R.; Smith, M. L.; Gourdon, D.; Vogel, V. *Nano Lett.* **2009**, *9*, 4158−4167.
(105) Lee, H.; Jang, Y.; Seo, J.; Nam, J. M.; Char, K. *ACS Nano* **2011**, *5*, 5444−5456.
(106) Berg, M. C.; Yang, S. Y.; Hammond, P. T.; Rubner, M. F. *Langmuir* **2004**, *20*, 1362−1368.
(107) Werner, S.; Huck, O.; Frisch, B.; Vautier, D.; Elkaim, R.; Voegel, J. C.; Brunel, G.; Tenenbaum, H. *Biomaterials* **2009**, *30*, 2291−2301.
(108) Tsai, W. B.; Chen, R. P. Y; Wei, K. L.; Chen, Y. R.; Liao, T. Y.; Liu, H. L.; Lai, J. Y. *Acta Biomater.* **2009**, *5*, 3467−3477.
(109) Chua, P. H.; Neoh, K. G.; Kang, E. T.; Wang, W. *Biomaterials* **2008**, *29*, 1412−21.
(110) Thompson, M. T.; Berg, M. C.; Tobias, I. S.; Lichter, J. A.; Rubner, M. F.; Van Vliet, K. J. *Biomacromolecules* **2006**, *7*, 1990−1995.
(111) Schneider, A.; Bolcato-Bellemin, A.-L.; Francius, G.; Jedrzejwska, J.; Schaaf, P.; Voegel, J.-C.; Frisch, B.; Picart, C. *Biomacromolecules* **2006**, *7*, 2882−2889.
(112) Chluba, J.; Voegel, J. C.; Decher, G.; Erbacher, P.; Schaaf, P.; Ogier, J. *Biomacromolecules* **2001**, *2*, 800−805.
(113) Jessel, N.; Schwinté, P.; Falvey, P.; Darcy, R.; Haïkel, Y.; Schaaf, P.; Voegel, J.-C.; Ogier, J. *Adv. Funct. Mater.* **2004**, *14*, 174−182.
(114) Ma, L.; Zhou, J.; Gao, C.; Shen, J. *J. Biomed. Mater. Res., Part B* **2007**, *83*, 285−292.
(115) Almodovar, J.; Bacon, S.; Gogolski, J.; Kisiday, J. D.; Kipper, M. J. *Biomacromolecules* **2010**, *11*, 2629−2639.

(116) Macdonald, M. L.; Rodriguez, N. M.; Shah, N. J.; Hammond, P. T. *Biomacromolecules* **2010**, *11*, 2053−2059.
(117) De Cock, L. J.; De Koker, S.; De Vos, F.; Vervaet, C.; Remon, J. P.; De Geest, B. G. *Biomacromolecules* **2010**, *11*, 1002−1008.
(118) Vodouhe, C.; Schmittbuhl, M.; Boulmedais, F.; Bagnard, D.; Vautier, D.; Schaaf, P.; Egles, C.; Voegel, J. C.; Ogier, J. *Biomaterials* **2005**, *26*, 545−554.
(119) Muller, S.; Koenig, G.; Charpiot, A.; Debry, C.; Voegel, J.; Lavalle, P.; Vautier, D. *Adv. Funct. Mater.* **2008**, *18*, 1767−1775.
(120) Dierich, A.; Le Guen, E.; Messaddeq, N.; Stoltz, S.; Netter, P.; Schaaf, P.; Voegel, J.-C.; Benkirane-Jessel, N. *Adv. Mater.* **2007**, *19*, 693−697.
(121) Nadiri, A.; Kuchler-Bopp, S.; Mjahed, H.; Hu, B.; Haikel, Y.; Schaaf, P.; Voegel, J. C.; Benkirane-Jessel, N. *Small* **2007**, *3*, 1577−1583.
(122) Paralkar, V. M.; Hammonds, R. G.; Reddi, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 3397−401.
(123) van den Beucken, J. J.; Walboomers, X. F.; Boerman, O. C.; Vos, M. R.; Sommerdijk, N. A.; Hayakawa, T.; Fukushima, T.; Okahata, Y.; Nolte, R. J.; Jansen, J. A. *J. Controlled Release* **2006**, *113*, 63−72.
(124) Macdonald, M. L.; Samuel, R. E.; Shah, N. J.; Padera, R. F.; Beben, Y. M.; Hammond, P. T. *Biomaterials* **2011**, *32*, 1446−53.
(125) Crouzier, T.; Szarpak, A.; Boudou, T.; Auzely-Velty, R.; Picart, C. *Small* **2010**, *6*, 651−662.
(126) Crouzier, T.; Sailhan, F.; Becquart, P.; Guillot, R.; Logeart-Avramoglou, D.; Picart, C. *Biomaterials* **2011**, *32*, 7543−54.
(127) Doshi, N.; Swiston, A. J.; Gilbert, J. B.; Alcaraz, M. L.; Cohen, R. E.; Rubner, M. F.; Mitragotri, S. *Adv. Mater.* **2011**, *23*, H105−H109.
(128) Swiston, A. J.; Cheng, C.; Um, S. H.; Irvine, D. J.; Cohen, R. E.; Rubner, M. F. *Nano Lett.* **2008**, *8*, 4446−53.
(129) Ren, K.; Fourel, L.; Gauthier-Rouviere, C.; Albiges-Rizo, C.; Picart, C. *Acta Biomater.* **2010**, *6*, 4238−48.
(130) Lutolf, M. P.; Weber, F. E.; Schmoekel, H. G.; Schense, J. C.; Kohler, T.; Muller, R.; Hubbell, J. A. *Nat. Biotechnol.* **2003**, *21*, 513−8.
(131) Nichols, J. E.; Cortiella, J.; Lee, J.; Niles, J. A.; Cuddihy, M.; Wang, S.; Bielitzki, J.; Cantu, A.; Mlcak, R.; Valdivia, E.; Yancy, R.; McClure, M. L.; Kotov, N. A. *Biomaterials* **2009**, *30*, 1071−1079.
(132) Lee, J.; Kotov, N. A. *Small* **2009**, *5*, 1008−1013.
(133) Guillame-Gentil, O.; Semenov, O.; Roca, A. S.; Groth, T.; Zahn, R.; Voros, J.; Zenobi-Wong, M. *Adv. Mater.* **2010**, *22*, 5443−5462.
(134) Broderick, A. H.; Azarin, S. M.; Buck, M. E.; Palecek, S. P.; Lynn, D. M. *Biomacromolecules* **2011**, *12*, 1998−2007.
(135) Shah, N. J.; Macdonald, M. L.; Beben, Y. M.; Padera, R. F.; Samuel, R. E.; Hammond, P. T. *Biomaterials* **2011**, *32*, 6183−6193.
(136) Mertz, D.; Vogt, C.; Hemmerle, J.; Mutterer, J.; Ball, V.; Voegel, J. C.; Schaaf, P.; Lavalle, P. *Nat. Mater.* **2009**, *8*, 731−735.
(137) Reisch, A.; Hemmerle, J.; Chassepot, A.; Lefort, M.; Benkirane-Jessel, N.; Candolfi, E.; Mesini, P.; Letscher-Bru, V.; Voegel, J. C.; Schaaf, P. *Soft Matter* **2010**, *6*, 1503−1512.
(138) Brown, X. Q.; Ookawa, K.; Wong, J. Y. *Biomaterials* **2005**, *26*, 3123−9. Wipff, P. J.; Majd, H.; Acharya, C.; Buscemi, L.; Meister, J. J.; Hinz, B. *Biomaterials* **2009**, *30*, 1781−1789.
(139) Kirchhof, K.; Andar, A.; Yin, H. B.; Gadegaard, N.; Riehle, M. O.; Groth, T. *Lab Chip* **2011**, *11*, 3326−3335.
(140) Boulmedais, F.; Tang, C. S.; Keller, B.; Voros, J. *Adv. Funct. Mater.* **2006**, *16*, 63−70. Wood, K. C.; Zacharia, N. S.; Schmidt, D. J.; Wrightman, S. N.; Andaya, B. J.; Hammond, P. T. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2280−2285.
(141) Guillaume-Gentil, O.; Akiyama, Y.; Schuler, M.; Tang, C.; Textor, M.; Yamato, M.; Okano, T.; Voros, J. *Adv. Mater.* **2008**, *20*, 560.
(142) Crouzier, T.; Fourel, L.; Boudou, T.; Albiges-Rizo, C.; Picart, C. *Adv. Mater.* **2011**, *23*, H111−8.
(143) Volodkin, D. V.; Delcea, M.; Mohwald, H.; Skirtach, A. G. *ACS Appl. Mater. Interfaces* **2009**, *1*, 1705−10.
(144) Volodkin, D. V.; Madaboosi, N.; Blacklock, J.; Skirtach, A. G.; Mohwald, H. *Langmuir* **2009**, *25*, 14037−43.
(145) Fischer, D.; Li, Y. X.; Ahlemeyer, B.; Krieglstein, J.; Kissel, T. *Biomaterials* **2003**, *24*, 1121−1131.
(146) Kean, T.; Thanou, M. *Adv. Drug Delivery Rev.* **2010**, *62*, 3−11.
(147) Brunot, C.; Ponsonnet, L.; Lagneau, C.; Farge, P.; Picart, C.; Grosgogeat, B. *Biomaterials* **2007**, *28*, 632−40.
(148) Kerdjoudj, H.; Berthelemy, N.; Rinckenbach, S.; Kearney-Schwartz, A.; Montagne, K.; Schaaf, P.; Lacolley, P.; Stoltz, J. F.; Voegel, J. C.; Menu, P. *J. Am. Coll. Cardiol.* **2008**, *52*, 1589−1597.
(149) Meng, S.; Liu, Z. J.; Shen, L.; Guo, Z.; Chou, L. S. L.; Zhong, W.; Du, Q. G.; Ge, J. *Biomaterials* **2009**, *30*, 2276−2283.
(150) Schultz, P.; Vautier, D.; Richert, L.; Jessel, N.; Haikel, Y.; Schaaf, P.; Voegel, J. C.; Ogier, J.; Debry, C. *Biomaterials* **2005**, *26*, 2621−2630.
(151) De Cock, L. J.; Lenoir, J.; De Koker, S.; Vermeersch, V.; Skirtach, A. G.; Dubruel, P.; Adriaens, E.; Vervaet, C.; Remon, J. P.; De Geest, B. G. *Biomaterials* **2011**, *32*, 1967−1977.
(152) Adriaens, E.; Remon, J. P. *Pharm. Res.* **1999**, *16*, 1240−1244.
(153) De Koker, S.; Naessens, T.; De Geest, B. G.; Bogaert, P.; Demeester, J.; De Smedt, S.; Grooten, J. *J. Immunol.* **2010**, *184*, 203−211.
(154) Wilson, J. T.; Cui, W.; Kozlovskaya, V.; Kharlampieva, E.; Pan, D.; Qu, Z.; Krishnamurthy, V. R.; Mets, J.; Kumar, V.; Wen, J.; Song, Y.; Tsukruk, V. V.; Chaikof, E. L. *J. Am. Chem. Soc.* **2011**, *133*, 7054−64.
(155) Hynes, R. O. *Science* **2009**, *326*, 1216−9.