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Concepts for Designing Tailored Thin Film Surfaces with Potential Biological Applications

Nicolás Eduardo Muzzio, Omar Azzaroni, Sergio E. Moya and Miguel Ángel Pasquale

Abstract

The tailoring of surfaces with nanostructured thin films, where interfacial properties can be controlled at the nanoscale, offers multiple possibilities for biological applications. The design of thin films with appropriate properties to induce desired biological responses at cell level requires the convergence of research from physics, chemistry, material science, biology, and medicine. Here, we will discuss the main surface properties that determine the behavior of isolated cells, cell colonies, and tissues interacting with a material. Surface roughness, morphological features, stiffness, wettability, chemical nature, and protein-surface interaction characteristics, as well as spatiotemporal heterogeneities, are expected to contribute to the desired biological performance of a material. A brief review in relation to thin films for biological applications will be presented. We will focus on examples in which basic rather simple processes play a key role in determining the triggering of a particular biological cell phenotype.

Keywords: tailored surfaces, biocompatible thin films, cell phenotype control, biocompatibility, multifunctionality

1. Introduction

The interfacial region of a real system interacting with the environment determines many of its properties [1]. The control of the structure, topography, composition, charge density and distribution, wettability, and the viscoelastic properties at the interface plays a key role in determining the ultimate characteristics of a material interacting with a biological system [2]. Thus, thin films and coatings can be used to obtain tailored interfaces with nanoscale controlled properties with the aim of generating systems with different complexity and specific functionalities of biological interest. This issue is of particular interest for engineering material surfaces with potential biomedical applications as implants or medical devices, or platforms for fundamental studies at the cell level [3–6].

Biological entities, such as proteins, prokaryote, or eukaryote cells, are expected to interact with a material when the material is in contact with biological fluids or tissues. In the case of the interaction of cells with an interface, the triggered biochemical signals that generate spatial and temporal changes in the interaction process should be considered [7]. Cells appear to be smart macromolecular systems

that trigger cooperative phenomena upon interaction with a substrate with particular physicochemical and biochemical properties.

Depending on its specific applications, a proposed biocompatible material is required to enhance or inhibit protein and cell adhesion. For instance, interfacial properties of materials for implants should promote tissue-material integration [8] and avoid bacterial infections [9]. Moreover, for many medical devices to act as barriers, antiadherence and antifouling characteristics are needed [10, 11].

In recent years, the combination of new and improved techniques for patterning a substrate [12, 13], spatially controlling the deposition of a material [14], supramolecular synthesis [15], as well as new assembling procedures to fabricate tunable films at the nanoscale [16, 17], has allowed the introduction and control of spatial and temporal, physicochemical and biological cues. It has been possible to wisely design materials to (i) modulate and study cell behavior and fate [18–21], (ii) deliver, release and sense bioactive molecules [22–25], and (iii) increase the biocompatibility and functionalities of materials and devices [26, 27]. In many situations, the tailored system properties depend on both the characteristics of the pristine substrate and those of the coating. Typical examples (**Figure 1**) are presented to show some design strategies, without pretending to be complete, as a large number of systems are possible: (a) a thin film is coated onto a rigid substrate to improve biocompatibility; (b) the thin film can be used to modulate the viscoelastic properties of the system; (c) a rigid substrate is modified by a coating containing species that can be internalized by
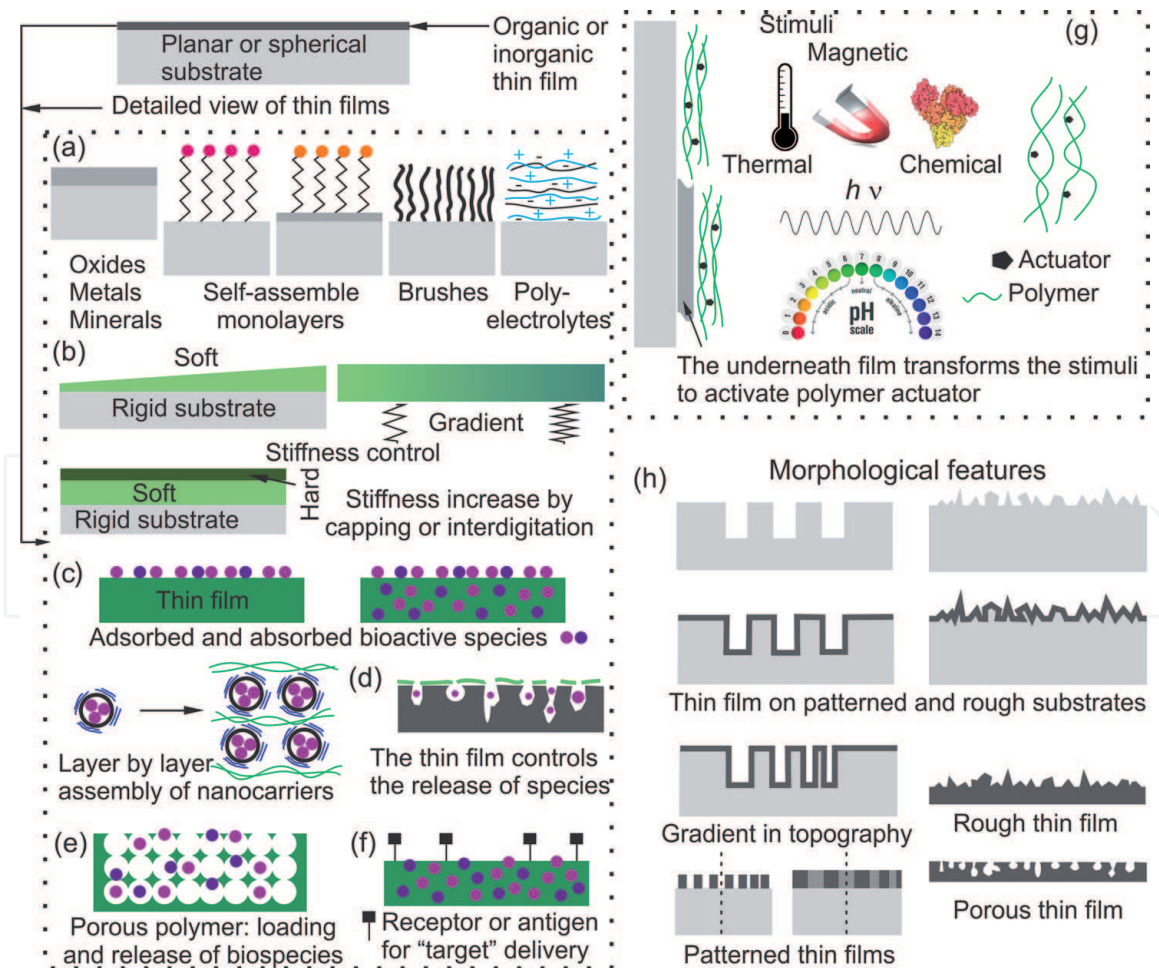

Figure 1.
Different strategies of thin film biological applications. (a) Organic and inorganic thin films that modify the interfacial properties toward cell behavior. (b) Stiffness modulation by organic thin films. (c) Thin film as cargo systems. (d) Thin film to control biomolecule release from a porous substrate. (e) Porous organic thin film for biomolecule release. (f) Thin film for "target" delivery. (g) Responsible thin films under either a direct or indirect stimulus. (h) Morphological features that can be incorporated by a thin film.

cells or are released to the medium; (d) the thin film controls the release of molecules from a solid substrate underneath; (e) the thin film is a porous material capable of releasing drugs encapsulated in the pores; (f) the thin film is tailored with species interacting specifically with a target; (g) either an underneath or an upper thin film bearing actuator moieties are responsive under external stimuli that trigger a change in the properties of the system; and (h) nanotopographic characteristics with spatial control can be introduced in the substrate surface. The topographic features can be combined with a thin film, and physicochemical properties such as specific moieties, stiffness, charge, and wettability can be tuned.

In the next sections, we will first give a brief comment about the desired biocompatibility of new proposed materials and survey some materials to fabricate thin films for biological applications. Then, we will depict the material properties that affect cell behavior and conclude with some examples of thin films to modulate cell behavior.

2. Thin films of biological interest

Thin films with potential applications in biology need to be biocompatible. There are many definitions for biocompatibility, but generally it is meant to refer to a material that is expected to carry out a suitable response in the host biological environment without producing any undesirable effects [28]. Different assays are performed to test the biocompatibility of a material, including in vitro experiments of cell proliferation and adhesion, interaction with biological fluids, and in vivo performance evaluation of the material function in model animals and its inflammatory response [29].

Many of the thin film materials presented in this chapter have potential applications but require further research for good biocompatibility.

2.1 Inorganic thin films

A wide variety of inorganic thin films and coatings have been proposed to be used in biology, including pure metals, alloys, metallic compounds, carbon-based thin films, oxide layers, and ceramic materials [3].

2.1.1 Metals and derived compounds

Titanium and its alloys are the most important materials for biomedical applications, particularly as bulk metal with proper surface modifications. Titanium oxide (TiO_2) and titanium nitride (TiN) coatings are very appealing as they form bioinert layers, protect substrates from corrosion and promote desirable biological functionalities, such as osteoblast adhesion and proliferation and apatite formation.

Mesoporous titania surfaces and nanotubular titania layers are appealing for biomedical applications since both titanium and titania exhibit good biocompatibility and are commonly utilized for biomedical devices and implants. Pores in the mesoporous titania can be designed for the encapsulation of drugs for delivery, growth factors, and antibiotics that can either facilitate cell material interaction or inhibit the presence of bacteria.

Gold and platinum metallic coatings are also used as microelectrodes to monitor signals from neurons and evaluate the electrophysiological activity of the neuronal network. Platinum-based thin films are also employed for pacemakers, implanted defibrillators, stents, and hearing assist devices, due to their good electrical conduction and biocompatibility.

2.1.2 Bioceramics and carbon-based materials

Bioceramic thin films are used for coating implants and prostheses, as they exhibit good corrosion resistance and better bioactivity than the metal substrate. Bioceramic thin films deposited on Ti prostheses yield better integration and bone growth in comparison with the bare metal. Some bioceramics utilized in patients are bioinert, for example, oxide and silicon ceramics. Other ceramics are resorbable, as is the case of calcium phosphates, and others are bioactive, such as bioglasses and hydroxyapatite.

Carbon-based materials, that is, diamond-like and carbon nitride thin films are potential materials for coating substrates for biological applications as biomedical devices. Diamond-like carbon shows lower platelet adhesion than carbon nitride. Despite this, carbon nitride thin films are able to yield harder coatings.

Unlike diamond-like and carbon nitride thin films that are amorphous carbon materials, nanocrystalline diamond thin films allow the tuning of their crystalline structure and can mimic the surface roughness of bone. These films also provide high chemical and corrosion resistance, making them very appealing for biological applications.

Carbon nanotubes and graphene sheets are promising materials for biomedical applications [30], presenting new opportunities due to their unique mechanical, electrical, and optical properties. Furthermore, carbon-based nanomaterials can mimic the microenvironment provided by the biological extracellular matrix [30]. These systems have been extensively studied as drug/gene delivery vehicles for anticancer treatments.

It has also been reported that graphene can favor the osteoblast linage of mesenchymal stem cells (MSCs) [31] and increases the rate of human MSCs osteogenic differentiation by a similar magnitude to bone morphogenic protein, BMP-2 [32].

2.2 Organic thin films and hybrid systems

Organic thin films, particularly those of natural origin, are very appealing for creating a biocompatible interface and are potentially suitable for biological applications. The drawback of these materials is their poor mechanical properties, as they resemble soft biological tissues. Soft materials are characterized by a limited cell adherence. Thus, they are often modified to fulfill the requirements of their intended application.

Examples of polymers used for thin film fabrication include the following:

1. Extracellular matrix components. For instance, collagen, fibronectin and laminin, growth factors and glycosaminoglycans, among others.

2. Zwitterionic polymers, which display an equal number of cationic and anionic groups making them hydrophilic and antifouling.

3. Polyethylene glycol-modified polymers (PEGylated polymers) and hyperbranched polymers with oligosaccharide surfactant hydrophilic moieties, which result in antifouling coatings.

4. Thermoresistive polymers derived from poly(N-isopropyl acrylamide) (PNIPAM) [33].

5. Polymers employed in the fabrication of devices (such as biosensors), used to prevent nonspecific adhesion of biological materials forming hydrophilic/
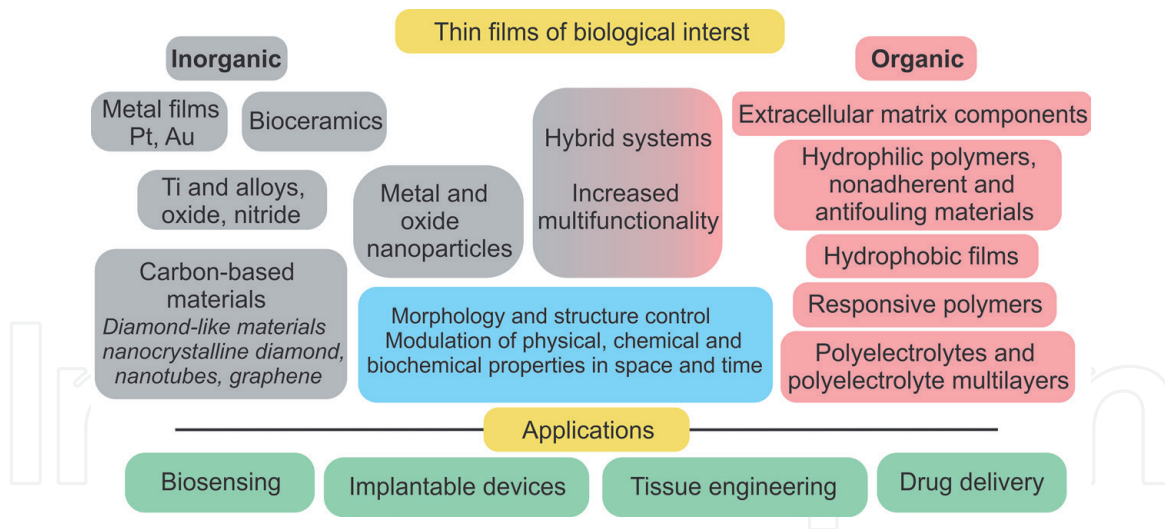
Figure 2.
Classification of materials for thin film coatings and applications.

hydrophobic layers, namely polydimethylsiloxane (PDMS), polyethylene (PE), perfluoropolyethylene (PFPE), polyimide (PI), parylene C, polyetheretherketone (PEEK), cyclic olefin/copolymer, poly(methyl methacrylate) (PMMA), polypropylene (PP), and polystyrene (PS) [34].

6. Polyelectrolyte multilayers (PEMs) of either synthetic or natural polyelectrolytes assembled by the versatile layer-by-layer (LbL) techniques driven by different molecular forces, that is, electrostatic or hydrogen bonding. The LbL technique allows the combination of different materials as building blocks and a wide variety of substrates can be coated.

Hybrid nature-inspired inorganic/organic materials have been fabricated, that is, layered systems can include metallic nanoparticles and nanotubes, mesoporous materials with organic moieties, hydrogels with in situ synthesized nanoparticles, etc. These systems can benefit from the multiple functionalities that can be carried by the nanomaterials to enhance gene delivery [35], imaging [36], and alternative disease treatments [37]. Furthermore, meso- and nanostructured titanium-based materials can be used to fabricate hybrid static and dynamic systems that include bioactive molecules as well as polymeric films to increase the material functionalities [21]. This approach is suitable to tackle the important issue of the miniaturization of medical devices.

A scheme of the classification of materials utilized to fabricate thin films of biological interest is depicted in **Figure 2**.

3. Thin film properties for tuning cell behavior

Living cells in an organism are sensitive to the environmental properties defined by neighboring cells and the extracellular matrix (ECM) [38]. Proteins play a key role in the cell sensing process of the microenvironment characteristics. For instance, cells are able to sense the viscoelastic and topographic properties of the environment through transmembrane proteins such as integrins [39]. Furthermore, growth factors, hormones, antibodies, vitamins, and other bioactive molecules can interact with specific cell receptors and induce a certain cell phenotype [40].

In general, cells in contact with a substrate interact with it through proteins deposited on the substrate. These proteins come from biological fluids or are

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produced by the cell itself upon interaction with the substrate [41]. Furthermore, in most of the experimental conditions employed for cell/material interaction studies, cells are cultured in medium supplemented with fetal bovine serum, and therefore, serum proteins interact with the substrate moieties. Among these proteins, bovine serum albumin (BSA) and fibronectin (FN) are usually considered as an example of nonadherent and adherent protein models, respectively.

Cell adhesion is the first step of many physiological and pathological processes such as wound healing, bacterial infections, and tumor progression. Eukaryote cell adhesion is possible if the intracellular forces exerted by stress fibers are balanced by the substrate stiffness [42]. The effect of the substrate stiffness is cell-type dependent, while adhesion of some cell lines is sensitive to changes in the substrate mechanical properties, other cells are less influenced [43]. Cells are able to sense the forces exerted by the environment and transduce them into biochemical signals; the study of this process is relatively recent and can profit from new actuator micro-devices such as microelectromechanical systems (MEMs) constructed with new biomaterials and methods to avoid toxicity [44].

On the other hand, cells can sense the stiffness of objects that are not in direct contact, depending on the compliant polymer thickness and its physicochemical properties [45]. Thus, cell adhesion can be modulated by depositing a compliant soft polymer upon a stiff support with controlled topography and by varying the thickness of the soft polymer [46]. Cells sense the stiffness that is the result of the combination of the stiffness of both the upper soft polymer and the rigid substrate underneath, namely "complex stiffness."

3.1 Substrate physicochemical properties

The cell adhesion characteristics and subsequent cell functionalities will depend strongly on the physicochemical properties of the biomaterial surface. In the next paragraphs, a brief survey of these properties will be given.

Wettability: Cell adhesion characteristics are optimal for surfaces with moderate wettability, that is, a contact angle of about 85°. It has been suggested that this behavior is due to the proper state of the adhesion-mediating ECM proteins that allow the interaction of cell receptors with specific protein motifs.

For highly hydrophobic surfaces, the amount of adsorbed protein is relatively large, but the intra-protein interaction and the protein/substrate interaction are high and may produce denaturing or hamper remodeling from cells.

On more polar hydrophilic surfaces, proteins adsorb in relatively low concentrations diminishing inter-protein interactions and preserving their native state. However, it has been observed that on highly hydrophilic surfaces, cell adhesion is limited and even completely unviable. In this case, the adhesion proteins are labile as relatively weak forces take place. This would cause the detachment of cells particularly when the monolayer density is high [2].

Surface charge: The charge of the coated substrate is an important factor for cell colony spreading. There are some research works that indicate that positively charged surfaces are more efficient toward cell adhesion than negatively charged surfaces. This fact is explained by the negative charge of most of the cell adhesion-mediating ECM species that would exhibit preferential adsorption at positively charged interfaces.

It has been reported that for UV-irradiated polytetrafluoroethylene in a NH3 atmosphere, the generated positive charge interacts synergistically with oxygen-containing groups, and the adhesion of mouse 3T3 fibroblast, human umbilical vein endothelial cells, and human embryonic kidney cells is stimulated [47–49].

Other works reported that the presence of carboxylate groups added to a copolymer of poly(DL-lactic acid) and poly(ethylene oxide) enhances rat aortic smooth muscle cells in comparison to a noncarboxylated surface [50]. The carboxylate groups may not only bring negative charge to the interface but they also increase its wettability to values suitable for cell adhesion [51].

It has been observed that bovine aortic endothelial cells adhere with a larger spreading area when the modified substrate bears a larger number of positively charged amine groups than negative carboxylate ones, both attached to self-assembled monolayers of alkanethiols [52]. In this work, the authors reported that the amount of osteopontin proteins adsorbed on both modified surfaces was very similar, thus they suggested that the orientation and geometrical conformation of osteopontin was more suitable for cell adhesion to the amino group-containing positive surface.

Substrate roughness and topography: In relation to these properties, it is convenient to distinguish three size scales: the macroroughness that involves features larger than 1 μm, the micro-/submicroroughness with features from 100 nm to 1 μm, and the nanoroughness with features below 100 nm.

Cell adhesion is insensitive to the macroroughness as cells are able to adhere to or between the surface irregularities that are larger than cells.

The micro-/submicroroughness significantly affects cell behavior as reviewed elsewhere [7]. Some studies have reported a positive influence of surface microroughness on cell adhesion, whereas in other studies, its influence has been shown to be negative. For instance, rat osteoblasts on microporous surface of titanium dental implants exhibited an increased average cell spreading area in comparison with flat surfaces [53]. Similarly, human osteoblast-like MG-63 cells cultured on Ti substrates with microroughness showed a more differentiated phenotype as a large expression of alkaline phosphatase (ALP), osteocalcin, and the faster production of an osteogenic environment were observed [54].

In contrast, a Ti-based alloy with microroughness exhibited poorer adhesion characteristics and decreased proliferation of MG-63 cells than flat surfaces [54].

These contradictory results are partially caused by the lack of systematic research work on the effect of micro-/submicroroughness, in part due to the ambiguity of the parameters used to characterize the surface roughness.

Finally, the nanoroughness of a biomaterial surface has been reported to have a beneficial influence on cell adhesion in a large number of papers. This fact occurs because nanoroughness resembles many aspects of the ECM morphological features. It appears that the proteins that induce cell adhesion adsorb on the biomaterial surface with the appropriate conformation for presenting specific sites, that is, Arg-Gly-Asp, to cell membrane receptors [2].

Substrate stiffness: The stiffness of the substrate plays a key role in cell function. For instance, by varying the elastic modulus of the polymer-coated substrate from about 0 to 500 kPa, the trigger of distinct cell functions has been observed and mechanistically described in relation to the forces exerted by cells [55]. Moreover, it has been reported that collagen cross-linking can modulate tissue stiffness to enhance integrin-mediated cell adhesion and induce the invasion by breast tumor cells [56].

Synthetic polymers such as polyacrylamide (PA) with modified stiffness have been utilized to study MSC differentiation. These cells differentiate into neurons on soft PA gels, myoblast on gels of intermediate stiffness and bone cells on stiffer gels [57].

PEM mechanical properties can be improved by adding layers of "hard" polyelectrolytes [58], properly changing the pH and the ionic strength during assembling [59], by cross-linking the layers [60], or incorporating nano-objects into the multilayer structure [20, 61]. In these cases, some of the reagents employed may be toxic and add additional synthesis steps.

3.1.1 Modulation of cell adhesion by thin film thermal annealing

In recent papers, we reported about the effect of thermal annealing of PEMs on the adhesion of different cell lines [62]. We employed polycationic terminated PEMs made up of 15 layers, (polycation/polyanion)₇polycation: poly-L-lysine (PLL)/alginate (Alg); PLL/dextran sulfate (DEX) and chitosane (Chi)/hyaluronic acid (HA). By treating PLL/Alg and PLL/Dex PEMs at 37°C for 1–3 days, we reported an enhancement of cell adhesion. The cytoplasm spreading area of A549 and C2C12 cells on unannealed PLL/Alg films is poor, while cells seeded on annealed (PLL/Alg)₇PLL PEMs present an extended cytoplasm with well-defined focal contacts and large actin fibers, like cells seeded on glass. In contrast, annealed Chi/HA PEMs exhibit a hampered adhesion of A549, C2C12, BHK, and MC-3T3 cells [26]. The advantage of this strategy is the absence of toxic substances and the simplicity of the procedure.

Recently, a well-characterized PEM based on poly(4-styrenesulfonic acid) (PSS) and poly(diallyl dimethyl ammonium chloride) (PDADMAC) has been employed to demonstrate the dynamic nature of the interface interacting with cells and the key role played by the strength of the interaction of proteins with the substrate [63]. Cells are able to sense the stiffness of the substrate provided they attach to adhesion proteins that are adsorbed on the interface with sufficiently high interaction energy. In this work, the authors measured an abrupt change in cell adhesion by varying the number of deposited layers (PEM thickness) rather slightly but maintaining its roughness constant, pointing out that the stiffness of the substrate was not an important property in the observed adhesion behavior, but rather the competition of proteins from the culture medium with preadsorbed ones. For these experiments, the authors used PEMs with variable surface charge and protein radiolabeling.

Thermal annealing results in the reorganization of PEMs, the polyelectrolytes rearrange in the multilayers from a stratified organization to molecular complexes of polycations and polyanions, where the interaction between oppositely charged polyelectrolytes is maximized [64]. For the case of PLL/Alg PEMs (**Figure 3a** and **c**), thermal annealing increases the stiffness of the film, increases the contact angle to about 90°, augments the negative charge, and renders a relatively flatter surface. Nevertheless, for both preadsorbed FN from a solution of FN alone and from a solution of FN and BSA, thermal annealing of PLL/Alg PEMs increases the stability of adsorbed FN as demonstrated by gamma counting of radiolabeled proteins and protein exchange assays. Furthermore, circular dichroism studies indicated that upon annealing, there is likely a larger number of arginine-glycine-aspartate (RGD) groups from FN that are able to interact with cell membrane integrins to favor cell adhesion [65].

In the case of Chi/HA PEMs (**Figure 3b** and **c**), the changes in physicochemical properties are significantly smaller after annealing than for unannealed PLL/Alg PEMs. The interface remains hydrophilic, the contact angle decreases from 30 to 21°, the charge becomes slightly more negative, and the stiffness remains unchanged. Contrarily, there is a significant change in the root mean square (RMS) roughness for annealed Chi/HA PEMs compared with unannealed Chi/HA PEMs: the RMS roughness decreases to half the value measured for unannealed films. This topographic change is expected to cause different interactions of the PEM surface with cells after annealing, increasing the antiadherent properties. For Chi/HA PEMs, we also conclude that thermal annealing reduces the interactions between adhesion proteins and the PEM interface, the reverse effect of that observed for PLL/Alg PEMs.

One of our goals in this work was to relate the changes in cell adhesion upon annealing of PEMs to the deposition and stability of adhesion and nonadhesion
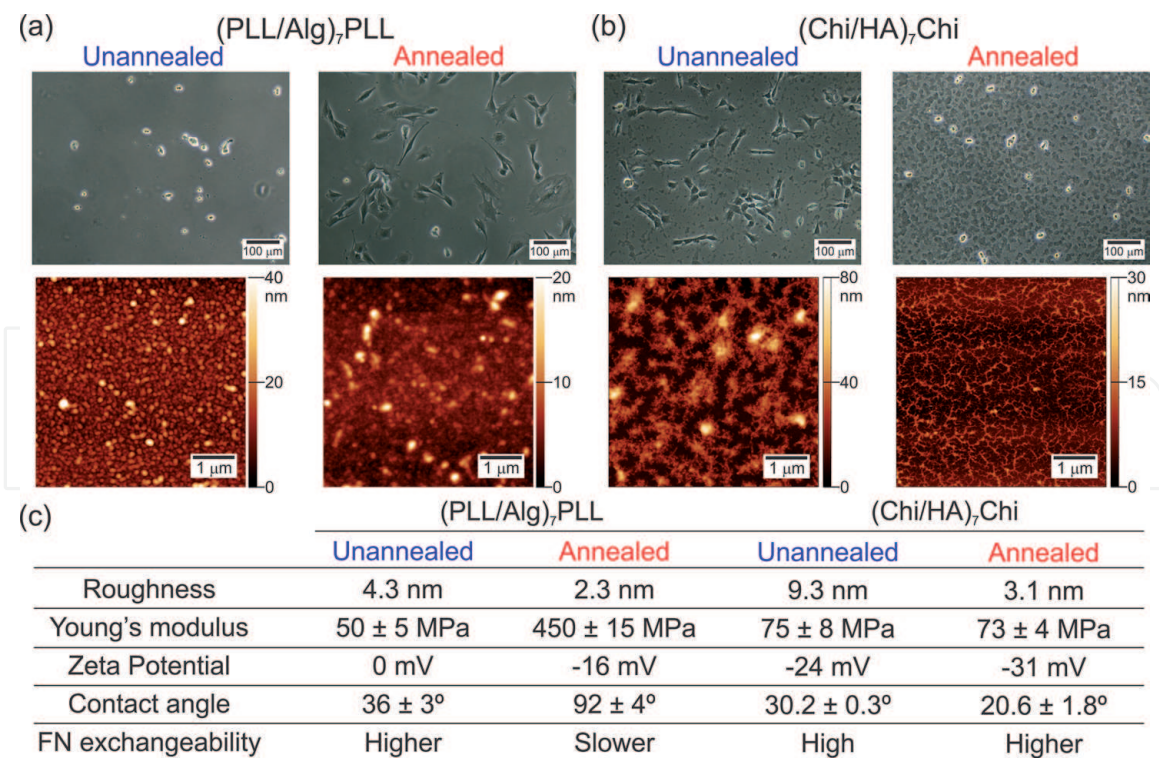
Figure 3.
Cell adhesion modulation on unannealed and annealed (PLL/Alg)₇PLL and (Chi/HA)₇Chi PEMs. (a)
Contrast phase images of A549 cells adhered to unannealed and annealed (PLL/Alg)₇PLL. The respective
atomic force microimages are included. (b) Cell adhesion and atomic force images for unannealed and annealed
(Chi/HA)₇Chi PEMs. (c) Physicochemical and fibronectin (FN) adsorption properties of unannealed and
annealed (PLL/Alg)₇PLL and (Chi/HA)₇Chi PEMs.

proteins and rationalize the effect of the changes in the physicochemical properties of PEMs on cell adhesion.

Cells are able to expand due to proper forces applied to the substrate for increasing cell cytoplasm tension. Thus, cells sense the increase in the Young's modulus of PLL/Alg films upon annealing provided adhesion proteins interact tightly with the PEM surface (**Figure 4a**). Upon annealing, both BSA and FN exhibit an increased interaction with the substrate. Moreover, FN adsorbed on annealed PLL/Alg adopts an elongated tertiary structure, favoring the exposure of RGD adhesive groups that enhance cell adhesion. For Chi/HA PEMs, results indicate a combined effect of a larger deposition of BSA (nonadhesive protein), and lower deposition of FN, the latter with an increased exchangeability, which renders the PEM surface unfavorable for stable interactions with integrins from the cell membrane (**Figure 4b**).

3.1.2 Modulation of cell adhesion by biologically inspired materials

Biologically inspired materials play a key role in the design of realistic platforms to modulate cell function. We proposed a novel biologically inspired supramolecular coating generated via one-step dip coating of the substrate in an aqueous solution of polyallylamine and phosphate anions [66]. We found selective cell adhesion, following the order in adhesion C2C12 myoblast > HeLa cells > MC-3 T3 preosteoblast, by varying the deposition time, that is, the thickness of the film from 20 to 120 nm (**Figure 5**) due to changes in the "complex stiffness". The proposed platform supports a cell-type dependent exponential proliferation rate, with viability tested during a month.

This phosphate-polyamine film can be used to coat either adherent or nonadherent substrates and perhaps even more interesting, relatively thick films (≈100 nm) that exhibit poor cell adhesion properties, irrespective of the cell line, recover their adhesive properties toward eukaryote cells upon annealing at 37° for 2–3 days
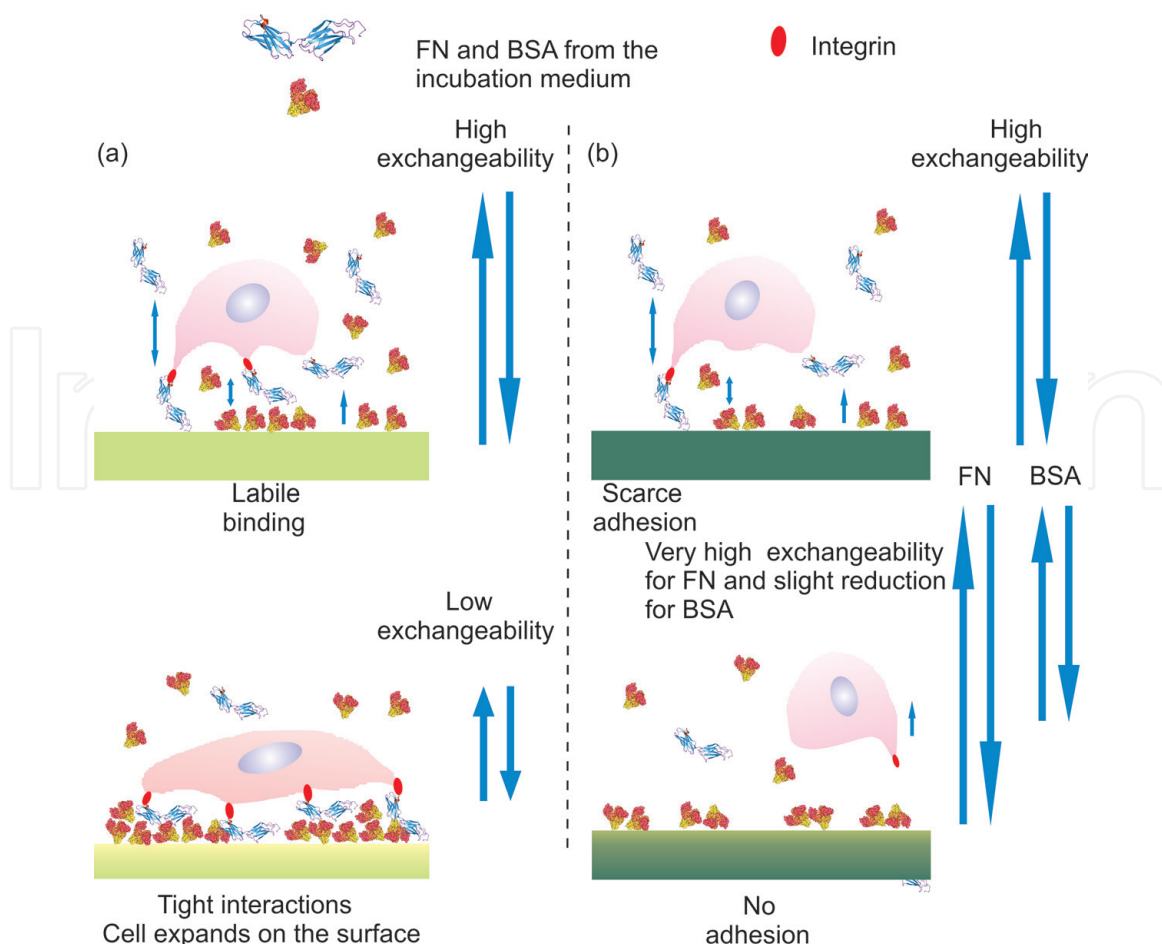
Figure 4.
Scheme of cell interaction with protein adsorbed on unannealed (top) and annealed (bottom) (PLL/Alg)$_7$*PLL (a) and (Chi/HA)*$_7$*Chi PEMs (b).*

(**Figure 5**). This effect was mainly explained by the increase in the film stiffness due to partial irreversible dehydration accompanied with an increase in the film roughness. Upon thermal annealing, the contact angle increases approaching about 70°, the surface charge remains negative, and the mass of FN adsorbed accessed by quartz crystal microbalance remains constant (**Figure 5d**).

3.2 Polymeric films with gradients

Cell behavior is markedly affected by the spatial distribution of mechanical and biochemical cues at the cell microenvironment. The overall cooperative result impacts on either physiological or pathological processes.

The fabrication of films with a gradual change in their swelling behavior has been obtained by gradual immersion of assembled PSS/PDADMAC multilayers into NaCl solutions [67]. In this work, the migration of smooth muscle cells (SMCs) at an appropriate cell density, in the direction of lowering hydration (relatively low swelling) has been observed. Furthermore, by time-controlled immersion in a solution of natural cross-linkers, a stable stiffness gradient on either free standing PEMs or assembled on silica substrates presenting differential cell adhesion has been reported [68].

A multilayer composed of poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH) grafted with photosensitive benzophenone was employed to generate stiffness gradient (55–140 MPa) by illuminating the assembled films with a gradient density filter that regulates the amount of UV light deposited onto the films [69]. Here, SMCs and MC-3T3 cells adhere and spread better on stiffer regions.
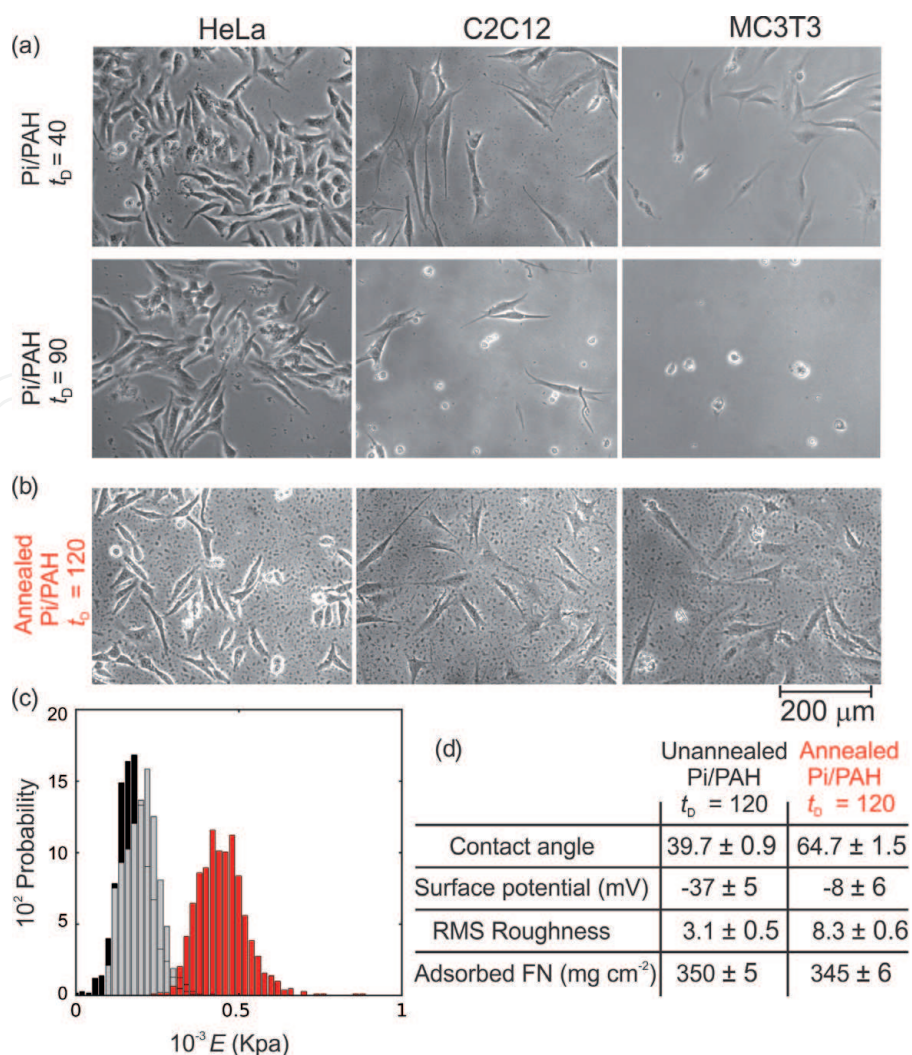
Figure 5.
Selective cell adhesion modulation on Pi/PAH-coated coverslips and the effect of thermal annealing at 37°C for 2–3 days. (a) Phase contrast images of HeLa, C2C12, and MC-3T3 cells seeded on unannealed Pi/PAH-coated substrates with t_D = 40 and 90 min, as indicated in the figure. (b) Contrast phase images of cells adhered to annealed Pi/PAH-coated substrates with t_D = 120 min. For C2C12 myoblasts and MC-3T3 preosteoblasts, cell adhesion characteristics improve in comparison with unannealed samples. (c) Stiffness (distribution of Young's modulus, E) for unannealed Pi/PAH samples with t_D = 40 min (black bars), t_D = 120 min (gray bars) and annealed Pi/PAH samples (red bars). (d) Some physicochemical properties of unannealed and annealed Pi/PAH-coated coverslips.

Other systems that include stiffness gradients [70] and concentration gradients of cell adhesive peptides or growth factors have been reported [71]. These concentration gradients are usually developed by microfluidic devices, allowing a spatially controlled surface arrangement of biological cues that are combined with physical ones to modulate cell fate.

We have recently reported on the post-assembly treatment of PLL/Alg PEMs with a temperature gradient between 10 and 50°C in samples 2 cm in length [19]. The resulting temperature gradient appears to be linear with the distance between sources at fixed temperature. A continuous change in the physicochemical properties appears to set in along the substrate upon the application of a thermal gradient. For the substrate region held at the highest temperature, C2C12 cell adhesion was roughly close to that observed on glass. In contrast, at the coldest region, cell adhesion was very poor. The most significant changes in the average cell spreading area were observed between 30 and 22°C (**Figure 6**). The application of a thermal gradient to locally modify the physicochemical properties of films as well as the adhesion characteristics toward different cell lines can be extended to other polymeric systems to increase the versatility of new materials for rapid testing of cell functionalities.
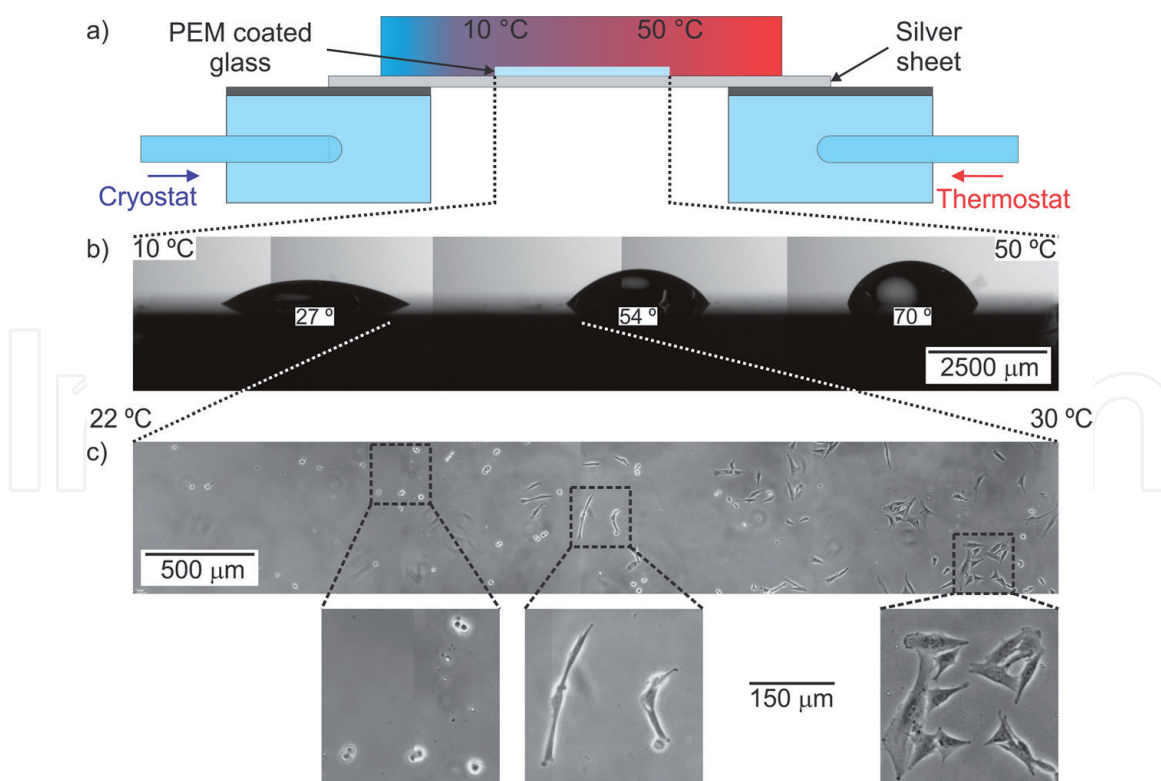
Figure 6.
Thermal gradient applied to (PLL/Alg)₇PLL PEM. (a) Scheme of the experimental setup for applying a thermal gradient. (b) Contact angle variation in the sample. (c) Spatial variation of cell adhesion characteristics.

4. Some hybrid multifunctional systems

The design of hybrid systems is motivated by the aim of profiting from the properties of the different materials composing them. For example, metal- and metallic oxide-derived thin films combined with polymeric layers that eventually contain bioactive molecules are useful to obtain multifunctionality.

Ti, its oxides, and alloys are widely used materials for biomedical applications. A detailed knowledge of the surface characteristics of Ti-based materials is needed for understanding the relation with their reactivity and biological performance [72]. Titanium oxide surfaces can be treated by chemical etching, sonochemical modifications or electrochemical treatment, to obtain hierarchically micro-/nanostructured porous surfaces [21]. These treatments enhance the photoactivity of the oxide layer. And, besides the inherent effect of the modified micro-/nanotopography on cell functions, for instance, the appropriate size of topographic features to efficiently promote integrin clustering, the porous materials can be loaded with bioactive molecules that promote prokaryote cell-material interaction [73].

By combining metallic surfaces with stimuli-responsive polyelectrolyte/polymer multilayers and supramolecular interacting systems, eukaryote and prokaryote cell behavior modulation has been achieved. A review of the application of these concepts is given in [21].

An example in relation to cell modulation behavior by a hybrid multifunctional system is provided in this section. The system is built up with a photocatalytically active TiO_2 film sonochemically nanostructured. Upon illumination, a photohole and a photoelectron are formed on the oxide surface as well as both H^+ and HO^-, one species prevailing over the other according to the composition of the interface. Thus, upon illumination for a certain time, a change in the pH at the interface is obtained. The system also includes a pH-sensitive high-amplitude

actuating polymer, a linear triblock terpolymer consisting of polybutadiene (B), poly(methacrylic acid) (MAA), and quaternized poly[2-(dimethylamino)ethyl methacrylate] (Dq) [74]. In aqueous solution, the system (BMAADq) forms self-assembled micelles (BCM) with a hydrophobic core and a pH-sensitive MAA shell, and a strong Dq corona. BCMs are positively charged and are assembled with PAA to fabricate the pH sensitive coating. The resulting TiO_2/BCM/PAA/BCM/PAA architecture is switched in response to the local changes in pH induced by the photocatalytic reactions on the oxide surface, resulting in a layer of variable thickness and stiffness. Thus, the authors achieve an efficient and controllable strategy to convert energy of electromagnetic illumination into a local pH variation that triggers the reversible response of the soft polymer. The same authors reported that the MC-3T3 cell migration is modulated in the proposed system. By local illumination of the film, cells are induced to migrate from softer to stiffer regions [75].

The strategy briefly commented above shows the capability of hybrid smart systems to modulate cell behavior by spatial and temporal control of physicochemical cues.

5. Conclusions

In this chapter, a number of materials and strategies employed to obtain inorganic, organic, and hybrid thin film functional systems with actual and potential applications in biosciences have been presented. We have depicted some materials of natural and synthetic origin employed to fabricate thin films to be used as platforms for fundamental cellular studies and potential applications. The system properties that affect cell functions and modulate its behavior and fate in a context approaching real situations have been also pointed out.

Furthermore, we briefly comment on some of our research works in relation to biocompatible materials based on polyelectrolyte assembly and post-assembly treatments to change the performance toward cell adhesion and proliferation.

The biocompatibility of these materials determines the ultimate success of a developed material. Despite the large number of wisely designed systems with tunable spatial and temporal properties, even at the nanoscale, the challenge to bring these advances to the clinic is a central issue and requires the convergence of basic and applied sciences as well as commercial and social considerations.

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