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# Polyelectrolyte Multilayers: A Versatile Tool for Preparing Antimicrobial Coatings

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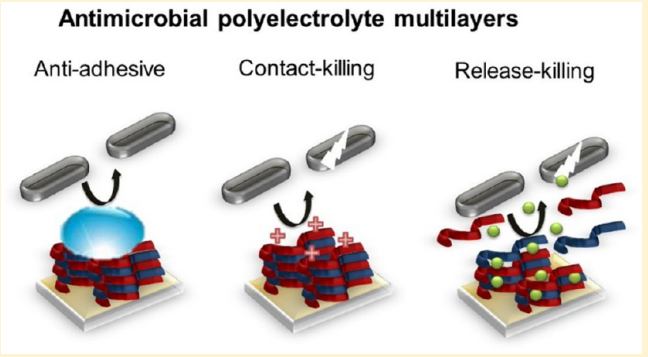
**ABSTRACT:** The prevention of pathogen colonization of medical implants represents a major medical and financial issue. The development of antimicrobial coatings aimed at protecting against such infections has thus become a major field of scientific and technological research. Three main strategies are developed to design such coatings: (i) the prevention of microorganisms adhesion and the killing of microorganisms (ii) by contact and (iii) by the release of active compounds in the vicinity of the implant. Polyelectrolyte multilayer (PEM) technology alone covers the entire wide-spread spectrum of functionalization possibilities. PEMs are obtained through the alternating deposition of polyanions and polycations on a substrate, and the great advantages of PEMs are that (i) they can be applied to almost any type of substrate whatever its shape and composition; (ii) various chemical, physicochemical, and mechanical properties of the coatings can be obtained; and (iii) active compounds can be embedded and released in a controlled manner. In this article we will give an overview of the field of PEMs applied to the design of antimicrobial coatings, illustrating the large versatility of the PEM technology.




## INTRODUCTION

Polyelectrolyte multilayers (PEMs) were introduced in 1991 by Decher[1] by extending to polyelectrolytes a technique initially developed by Iller[2] for colloidal particles. PEMs are obtained by the step-by-step deposition of polyanions and polycations interacting electrostatically with each other (Figure 1a).

This step-by-step deposition process of macromolecules was later extended to other types of interactions such as hydrogen bonding and even covalent bonding between macromolecules. PEMs can be constructed by dipping the substrate alternately in the different polyelectrolyte solutions, by spin-coating, or by spraying them.[3] Different physicochemical and mechanical properties can be obtained depending on the buildup conditions (pH, ionic strength) and the polyelectrolytes used. The growth of PEM films can be either linear or exponential. For linearly growing films, the thickness increases linearly with the number of deposition steps, and for the others, the thickness increases exponentially, at least during the first deposition steps. Whereas the thickness of the first films remains in the nanometer range per bilayer, the thickness of exponentially growing films can reach several micrometers after 10−15 bilayer deposition steps.[4] Linearly growing films are encountered for polyelectrolytes which interact strongly at each deposition step.[5] The resulting multilayer is thus a stratified architecture with no diffusion of the polyelectrolytes perpendicular to the film.[6] Poly(styrenesulfonate)/poly(allylamine) (PSS/PAH) constitutes a prominent example of this type of film. Exponentially growing PEMs are obtained for polyanions and polycations that interact weakly,[5] leading to much more hydrated and softer films than linearly growing ones.[7] This growth process is observed when at least one of the polyelectrolytes constituting the film diffuses in and out of the whole structure during each "bilayer" deposition step.[8] Hyaluronic acid/poly(L-lysine) (HA/PLL) is the most investigated exponentially growing multilayer. These films are of interest because they can be used as reservoirs for embedded proteins or enzymes.[9] A recent review of Caruso discusses how to engineer PEM films with appropriate physicochemical

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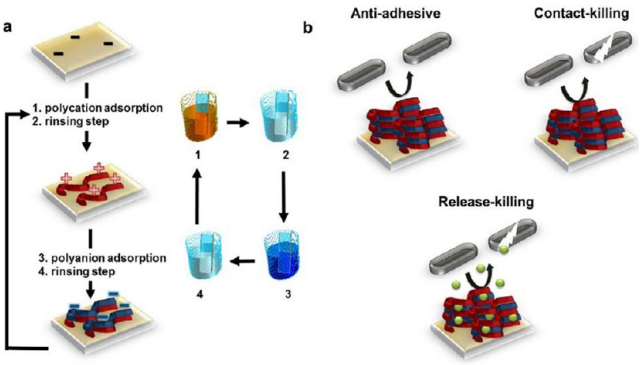


**Figure 1.** (a) Schematic representation of a polyelectrolyte multilayer (PEM) film buildup by successive adsorption steps of polycations and polyanions followed by rinsing steps using the dipping method. (b) Three main strategies were followed to design antimicrobial PEM: antiadhesive films inhibiting the close approach of pathogens, contact-killing films by exposing antimicrobial agents on the surface, and release-killing films delivering antimicrobial agents in the supernatant, with the last two strategies leading to the death of pathogens.

properties by making judicious choices of the assembly technology.[10] The great advantages of the method are that (i) it can be applied to almost any type of substrate whatever its shape and composition; (ii) various chemical, physicochemical, and mechanical properties of the coatings can be obtained; and (iii) active compounds can be embedded and released in a control manner. They thus represent a method of choice for developing antimicrobial coatings.

The prevention of pathogen colonization of medical implants constitutes a major medical and financial issue since nosocomial infections represent one of the most serious complications after surgery or critical care. The development of antimicrobial coatings aimed at protecting against such infections has thus become a major field of scientific and technological research. A wide range of strategies have been developed to design new antimicrobial coatings.[11] Various approaches based on the immobilization of antifouling polymers or bactericidal substances using self-assembled monolayers,[12] covalent attachment, or graft polymerization[13] have been explored and extensively reviewed. Chemical surface treatments are focused on preventing adhesion and killing or inhibiting the growth of pathogens before they settle on a surface. These strategies make use not only of the chemical,[14] mechanical,[15] and morphological properties of the coatings[16] but also of different active compounds such as antibiotics and other antibacterial compounds incorporated into the coatings.[17] Numerous surfaces and commercially used objects have already been coated with different antimicrobial agents showing all potential applications (Table 1). The PEM technology allows us to cover the entire widespread spectrum of functionalization possibilities. Three main kinds of PEM coatings have been developed to limit microbial colonization on material surfaces: adhesion-resistant, contact killing, and antimicrobial agent leaching coatings (Figure 1b). In this feature article, we will try to give an overview, often based on our own contribution, to the field of antimicrobial coatings related to PEMs. We will not cover the whole body of literature but rather will try to illustrate the different possibilities opened by PEM technology to address the different strategies mentioned above.

**Table 1. Examples of the Different Surfaces That Have Been Coated with PEM Films and Their Applications**[a]

| coated surfaces | antimicrobial agent | application | references |
|---|---|---|---|
| **Polymers** | | | |
| poly(dimethylsiloxane) | antiadhesive PEM | microfluidic devices | 18 |
| poly(ethylene terephthalate) | polycation | water purification | 19 |
| | antiseptic | textile | 20 |
| poly(L-lactic acid) | AgNP | tissue engineering | 21 |
| poly(propylene) | polycation | packaging | 22 |
| polystyrene films | polycation | laboratory plates | 23 |
| polyurethane | polycation | medical devices (catheters) | 24 |
| styrene-butadiene-styrene block copolymer | polycation | wound dressing | 25 |
| **Metals** | | | |
| copper | AgNP | marine coatings | 26 |
| stainless steel | polycation | marine coatings | 27 |
| | AgNP | orthopedic implants | 28 |
| | AMP | medicine application | 29 |
| titanium/titanium alloy | antibiotic | orthopedic implants | 30 |
| | AgNP | | 31 |
| **Fiber Mats** | | | |
| cellulose fibers | layered silicate/ polycation | healthcare product | 32 |
| | polycation | | 33 |
| nylon and silk fibers | AgNP | clothing product | 34 |
| polyacrylonitrile fibers | polycation | filtration, wound dressing, vascular graft | 35 |
| **Medical Devices** | | | |
| Biobrane | AgNP | biological dressing | 36 |
| catheter tube | AMP | fluid or gas administration | 37 |
| cortical bone | polycation | bone graft | 38 |
| cotton gauzes | AMP | wound dressing | 39 |
| gelatin sponge | antibiotic | adsorption of blood during invasive surgeries | 40 |
| intraocular lenses | antibiotic | intraocular lense implantation | 41, 42 |
| microfiltration membranes | antiadhesive PEM | drinking water treatment | 43 |
| suture materials | antibiotic | medical device coating | 42, 44 |

[a]AgNP, silver nanoparticles; AMP, antimicrobial peptides.

## STRATEGIES TO PREVENT BACTERIAL ADHESION

Because the first step in bacterial infection on implants is the bacterial adhesion on the substrate, the first strategy to create antimicrobial coatings that comes to mind is the design of substrates that prevent bacterial adhesion. Adhesion-resistant PEMs can be obtained in two different ways: (1) by inhibiting the close approach or the contact of the bacteria with the surfaces through the use of hydrophilic polymer-based PEM films[18,19,45,46] (Figure 2a) or (2) by using films with specific stiffnesses so that the bacteria do not adhere once entering into contact with the film (Figure 2b).[47,48]

To develop bacterial adhesion-resistant surfaces, one of the strategies consists of the use of hydrophilic polymers that can inhibit contact with or the close approach of bacteria to the surface due to the strong affinity of the polymers for water molecules. The simplest way to build hydrophilic PEMs is to


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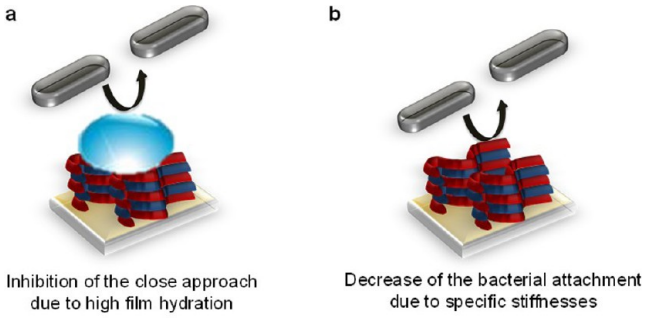


**Figure 2.** Scheme of the two strategies that have been used to build adhesion-resistant PEMs: (a) highly hydrated films prevent the adhesion of pathogens and (b) a range of specific stiffnesses of the films allow decreases in the attachment of pathogens.

use polyelectrolytes known for their high hydrophilicity. Heparin (HEP), a strong polyacid (with a $pK_a$ ranging from 0.5 to 1.5), and hyaluronic acid (HA), a weak polyacid (with a $pK_a$ of 2.9), are two ideal candidates known for this. As a polycation, chitosan (CHI), which is a weak polybase (with a $pK_a$ of 6.5), was also often chosen because it is the only known cationic polysaccharide that also possesses an intrinsic antibacterial property.[49] Because CHI is a weak polycation, the pH condition used to build the PEM plays a critical role in the antiadhesive properties of CHI/HEP films.[19] By varying the assembly pH from 2.9 to 6, the degree of ionization of CHI can be adjusted. At high pH values, CHI chains, with a low charge density, adopt loopy structures and tend to adsorb as thick layers. At low pH values, CHI chains adopt flatter structures, and the adsorbed layer is thinner. The surface composition of PEM films can thus be tuned by the assembly pH. The most hydrophilic PEM film, prepared at low pH, rich in HEP, entailed a better antiadhesive property whereas the most CHI-rich surfaces prepared at pH ∼6 had the most efficient antimicrobial (killing-effect) property against *E. coli*.[19] Our group investigated the effect of the ionic strength on the antiadhesive properties of CHI/HA films.[50] Compared to a bare glass substrate, CHI/HA films assembled at 0.15 M NaCl, pH 5, led to a decrease of about 80% in *E. coli* adhesion whereas only a 40% decrease was found when the multilayer was assembled at $10^{-2}$ M NaCl. The higher adhesion of bacteria under this last condition could be explained by the high rigidity of these thin films obtained at $10^{-2}$ M NaCl.

Another way to create highly hydrophilic PEMs is to use polyelectrolytes chemically modified by grafting hydrophilic chains or moieties. These modified polyelectrolytes are then incorporated into multilayers or can be deposited on top of a multilayer. In this way the hydrophilicity of the coating can be changed by varying the nature of the grafted chains and the grafting ratio. Adsorbed or grafted onto a surface, poly(ethylene glycol) (PEG) molecules act as a highly hydrated polymer layer reducing protein adsorption and bacterial adhesion.[51] In 2004, our group was the first to report the insertion of PEG into PEM in order to enhance its antiadhesive properties toward bacteria.[45] A PEG-functionalized poly(L-glutamic acid), PGA-*g*-PEG (Figure 3a), was synthesized and combined with poly(L-lysine) (PLL) to build PEM films. Composed of 45 ethylene glycol monomers, PEG chains were grafted at a ratio of 16% on PGA. $(PLL/PGA\text{-}g\text{-}PEG)_n$ multilayers were deposited on a PEM precursor film and were tested with respect to bacterial adhesion for 30 min. In comparison with the uncoated glass substrate, the adhesion of *E. coli* was reduced by 72% on PEM films composed of one PLL/PGA-*g*-PEG bilayer and by 92% on films terminated by three PLL/PGA-*g*-PEG bilayers (Figure 3b).

In order to minimize the nonspecific adhesion of a yeast, *Saccharomyces cerevisiae*, on poly(dimethylsiloxane) (PDMS)-based microfluidic devices, a pegylated poly(acrylic acid) (PAA-*g*-PEG) was adsorbed as the terminal layer on a PAA/poly(allylamine hydrochlroride) (PAH) or a PAA/poly(diallyldimethylammonium chloride) (PDADMAC) PEM film.[18] The PEG chains were constituted of 110 monomer units, and the grafting ratio was 20%. Compared to a bare PDMS substrate, a reduction of yeast cell adhesion of at least 2 orders of magnitude was found for the most efficient coating, i.e., a PAA-*g*-PEG-terminated PAA/PDADMAC film. Yet, it has to be noted that hydrated PAA/PAH and PAA/PDADMAC films already possess some antiadhesive properties when

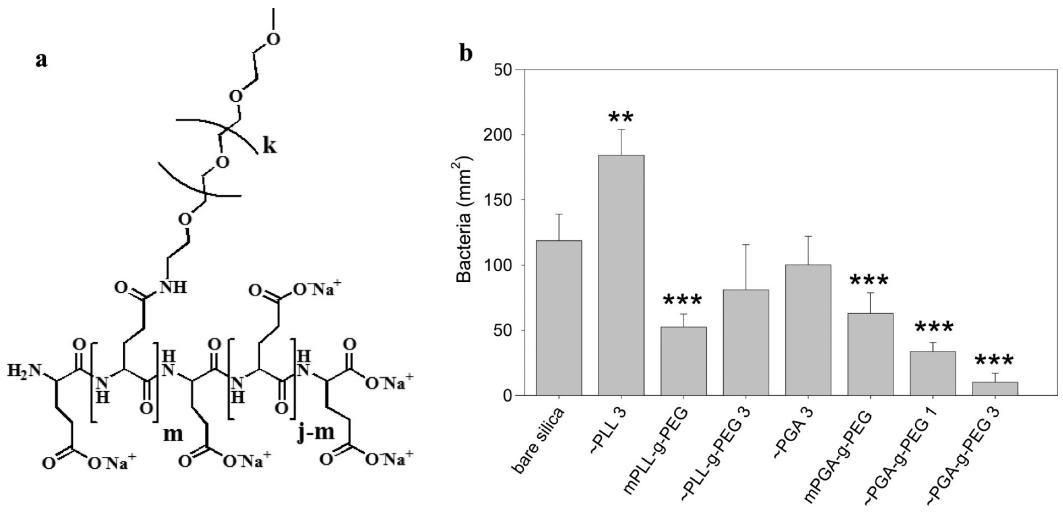


**Figure 3.** (a) Chemical structure of PGA-*g*-PEG, where $k \approx 45$, $j \approx 330$, and $m \approx 60$. (b) Bacterial adhesion per mm² on bare silica, mPLL-*g*-PEG = PLL-*g*-PEG monolayer, mPGA-*g*-PEG = $(PLL/PGA\text{-}g\text{-}PEG)_1$, and multilayer films ∼PLL 3 = Pre-$(PLL/PGA)_2$, ∼PLL-*g*-PEG 3 = Pre-PLL-$(PGA/PLL\text{-}g\text{-}PEG)_3$, ∼PGA 3 = Pre-$(PLL/PGA)_3$, ∼PGA-*g*-PEG 1 = Pre-$(PLL/PGA\text{-}g\text{-}PEG)_1$, and ∼PGA-*g*-PEG 3 = Pre$(PLL/PGA\text{-}g\text{-}PEG)_3$ with Pre = poly(ethylene imine)-$(PLL/PGA)_2$-PGA precursor film. Reprinted from ref 45. Copyright 2004 with permission from Elsevier.



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deposited on PDMS substrates.[18] This is attributed to their highly hydrated structure.

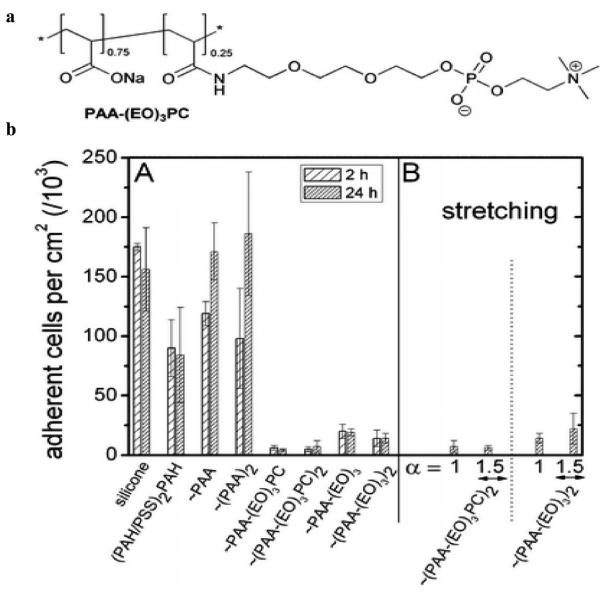


**Figure 4.** (a) Chemical structure of PAA-$(EO)_3$-PC with a 25% grafting ratio. (b) Adhesion of *C. albicans* onto a silicone sheet functionalized with one or two bilayers of (PAH/PAA), (PAH/PAA-$(EO)_3$-PC), and (PAH/PAA-$(EO)_3$) deposited onto a $(PSS/PAH)_2$ precursor film compared to the $(PAH/PSS)_2$PAH film (A) at rest and (B) under a stretching degree of 1.5. Reproduced from ref 52 with permission fromThe Royal Society of Chemistry.

As for eukaryotic cells, the elastic modulus of a substrate also seems to play a role in bacterial adhesion, but the results are still controversial. Polyelectrolyte multilayers make it easier to modulate the elastic modulus of a film without significantly changing its chemical surface properties. The first study reporting the influence of the elastic moduli on bacterial adhesion was performed by Lichter et al. in 2008 using PEMs.[47] The effective stiffness of PAH/PAA films was varied over several orders of magnitude (elastic modulus ranging from 1 to 100 MPa) by tuning the assembly pH. The bacterial attachment of *S. epidermidis* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria) increased with the stiffness of the PAH/PAA films independently of other surface characteristics such as surface roughness, surface interaction energy, or surface charge density. The elastic modulus of PEM can also be varied by cross-linking the films. This is most effective by using exponentially growing multilayers whose Young modulus can be varied over a wide range simply by changing the cross-linking degree. In this way Saha et al.[48] used PLL and HA modified by photoreactive vinylbenzyl groups (HAVB) to prepare photo-cross-linkable PEM films with different mechanical properties (an elastic modulus ranging from 30 to 150 kPa) under UV light illumination (Figure 5a). PLL/HAVB films were placed in contact for 1 h with Gram-positive and Gram-negative bacterial strains. While the growth of Gram-positive *Lactococcus lactis* was independent of the rigidity of PLL/HAVB PEM films, the growth of Gram-negative *E. coli* was slowed down on stiffer films compared to on softer ones. When incubated onto photopatterned PLL/HAVB films having a rigidity micropattern, a larger amount of *E. coli* was observed on the softer background and on the border between softer and stiffer regions. After image analysis in Figure 5b, we notice a greater number of adhered bacterial colonies on the non-cross-linked region (red patches) than on the photo-cross-linked regions (white patches) (Figure 5c). At first sight, these results seem to contradict the study described previously by Lichter et al.[47] This probably comes from the difference in the elastic modulus range studied. Indeed, the elastic moduli varied from 1 to 100 MPa for Lichter et al. and from 30 to 150 kPa for Saha et al.[48] In addition, the chemistry of PEM films is quite different: synthetic polyelectrolytes were used in the first study, and a polypeptide and a natural biopolymer were used in the second one. The chemical composition of the surface is an

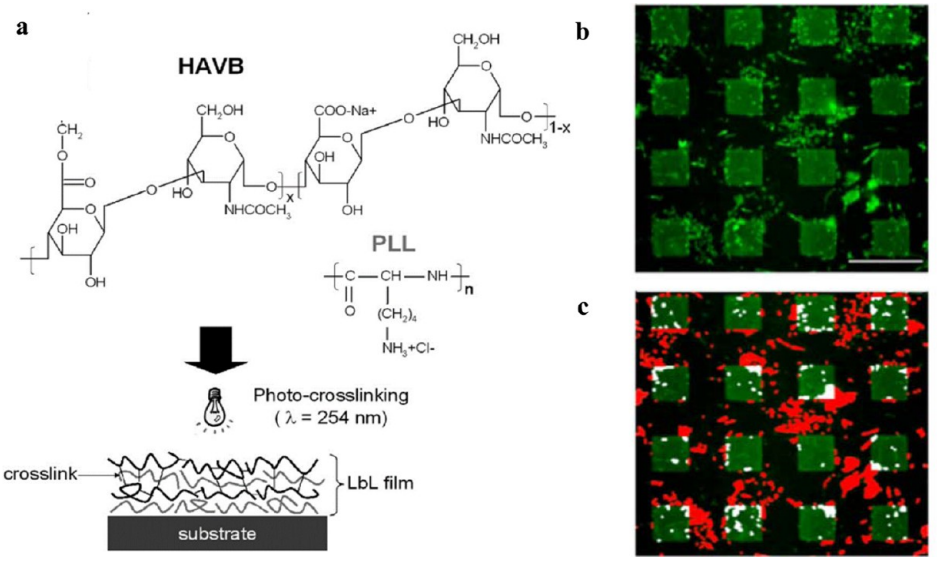


**Figure 5.** (a) Preparation of photo-cross-linked (PLL/HAVB) LbL films. (b) Microscopy epifluorescence image showing *E. coli* M6155 after 4.5 h of growth on photopatterned films; the lighter 20 × 20 $\mu m^2$ features correspond to stiffer photo-cross-linked regions while the dark background corresponds to a non-cross-linked film. The scale bar represents 40 $\mu$m. (c) Image analysis of the same area to discriminate the bacterial colonies adhered to the photo-cross-linked regions (white patches) and the non-cross-linked background (red patches). Reprinted with permission from ref 48. Copyright 2013 American Chemical Society.



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important aspect of the bacterial response. To conclude, the film stiffness seems to play an important role in the adhesion of bacterial cells. However, the influence of the stiffness on bacterial adhesion remains an open question, and PEMs could represent an ideal tool for further investigating this issue.

Adhesion-resistant PEMs have the advantage of preventing the first step in biofilm formation, i.e., pathogen adhesion, but pathogens in solution could stay alive and can further colonize another surface area.

## CONTACT-KILLING PEMS

Surrounded by a capsule which is mostly constituted of acidic polysaccharides, most bacterial cell membranes are negatively charged. Hence most antimicrobial polymers are positively charged to promote the interaction with the membrane. Due to cationic charges available on their surface, they inhibit bacterial proliferation by disrupting their membranes, leading to death. Contact-killing PEM films terminated by polycations were thus developed (Figure 6a).[22,27,32,33,53−58] Other contact-killing films incorporate other antimicrobial agents such as carbon nanoparticles[59,60] and enzymes[61] (Figure 6b). The design of these films is thus based on the ease of the PEM technology to control the surface charges and to incorporate almost any kind of molecule or particle in a multilayer.

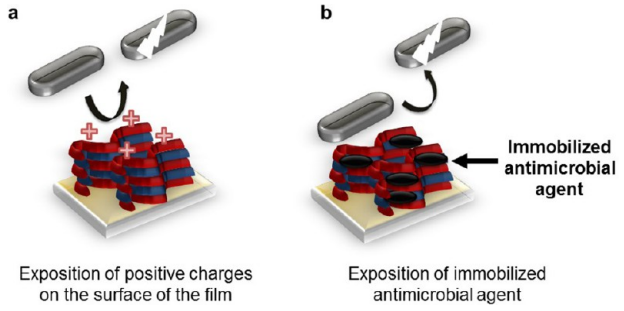


**Figure 6.** Scheme of the different strategies to obtain contact-killing PEMs: (a) highly positively charged surface and (b) immobilized antimicrobial agents, on the surface and inside the film, kill pathogens by contact.

The easiest way to create a multilayer with a positively charged surface is to terminate the film construction with a polycation. The advantage of using multilayers instead of a simple cationic layer to cover the substrate is (i) to render the coating substrate-independent and universal, i.e., less sensitive to small composition variations, and (ii) to have a reservoir of polycations of a controlled amount. As already mentioned, CHI is the only known polycationic polysaccharide (Figure 7a). Associated with other natural polymers such as pectin,[22] carrageenans,[55] HA,[56] or alginate,[32] CHI-based PEM films inhibited bacterial and fungal growth when a sufficient number of layers is reached. In particular, PEMs terminated by a CHI layer were reported to possess antibacterial properties which are explained by a larger number of amino groups available on the surface compared to films with a polyanion terminating layer.[55] The antibacterial properties of CHI/alginate[32] or CHI/pectin[57] films deposited on nanofibrous mats were slightly improved by binding a layered silicate, organic rectorite, with one of the polyelectrolytes during assembly. Although not antimicrobial, clay with a large specific surface area, such as organic rectorite, could immobilize the bacteria with the help of its excellent adsorption capacity. The antibacterial properties of CHI and rectorite nanocomposites result from the synergy between the adsorption and immobilization of the bacteria on the surface of the clay and the stronger interaction between amino groups and bacteria due to the accumulation of CHI on the surface of the clay.

Most often, there is almost an exact compensation between positive and negative charges of the polyelectrolytes and of ions constituting the film. After buildup, when using weak polyelectrolytes, the positive/negative charge ratio coming from polyelectrolytes can be tuned in the film simply by changing the pH of the supernatant solution. Along this line, the assembly and postassembly pH values of PEM films such as PAH/PSS and PAH/PAA were modified in order to expose

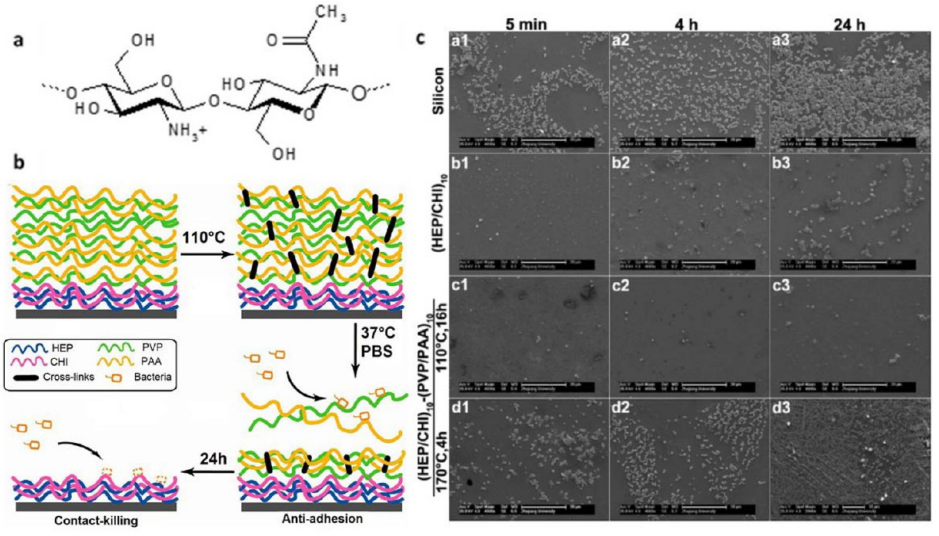


**Figure 7.** (a) Chemical structure of chitosan. (b) Schematic representation of the construction, cross-linking, degradation, and antibacterial properties of the $(HEP/CHI)_{10}$-$(PVP/PAA)_{10}$ multilayer film. (c) SEM images of water-borne assays of *S. aureus* adhesion on a silicon wafer (a1−a3), the $(HEP/CHI)_{10}$ multilayer film (b1−b3), and the $(HEP/CHI)_{10}$-$(PVP/PAA)_{10}$ multilayer films cross-linked at 110 °C for 16 h (c1−c3) or at 170 °C for 4 h (d1−d3). Scale bars are 20 $\mu$m. Reprinted with permission from ref 65. Copyright 2013 American Chemical Society.



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enough mobile cationic charges and to obtain antimicrobial activity against *S. epidermidis* and *E. coli*. At high pH, near or above their p$K_a$, PAH chains were incorporated with many uncharged amine groups into PEM films, revealing no contact-killing ability. By lowering the pH, these uncharged amines were protonated, creating sufficient positive charges to induce cell death.[53] By simple pH variation, PAH protonation can be varied, leading to a conformational change which allows exposing sufficient positive charges to obtain an antibacterial property.

Phenolic compounds are known to cause bacterial cell death through the disintegration of the cell membrane. They interact with the surface of the cell through van der Waals interactions of the phenyl ring and the hydrophobic tails of the lipids. Pinto et al. reported the use of poly(4-vinylphenol) (PVPh) assembled with PAH or PDADMAC at pH ranging from 10.5 to 12.5.[54] Both systems led to a maximum of *S. epidermidis* growth inhibition of about 60−70% at pH 10.5. Only a small antibacterial activity (maximum of 35% inhibition) was found against *E. coli*. The increase in antimicrobial efficacy at the lower assembly pH was likely due to the protonation of the phenol moiety that increased the mode of action of the multilayer.

Quaternary ammonium compounds (QAC) present the advantage of being permanently charged, independently of the solution pH, and are known to possess antimicrobial properties.[62] Thus, to improve the antibacterial activity of CHI, quaternized derivatives of CHI (QCHI) were synthesized and incorporated into PEM films.[56] These polyelectrolytes were not used as substrate coating but to build up PEM capsules, another major field of research in PEM. The degree of substitution of QCHI was demonstrated to be a crucial parameter in inhibiting bacterial growth. The higher degree of substitution led to the lowest survival of *E. coli* bacteria. Contact-killing QCHI/HA microcapsules, based on QCHI with the highest degree of substitution, show the most efficient effect on killing *E. coli*.[56] QCHI-terminated capsules appeared to be more effective at killing bacteria than HA-terminated ones. Bacteria appeared to be stuck on microcapsules, probably due to attractive electrostatic interactions between the positively charged capsules and the negatively charged bacteria.

Other polymeric quaternary ammonium salts have also been incorporated into PEM films.[27,58] The association of *N*,*N*-dodecyl,methyl-poly(ethylenimine) (DMLPEI) and PAA when built under optimal conditions leads to the growth inhibition of both Gram-positive and Gram-negative bacteria as well as of an influenza A/WSN (H1N1) virus.[58] Following Rubner and co-workers' study,[53] Wong et al. demonstrated the importance of the pH assembly by making use of the fact that PAA is a weak polyacid.[58] The bactericidal activity of DMLPEI/PAA films against *S. aureus* increases as the pH of the PAA solution used for assembly is lowered. This is consistent with the current view that mobile positive charges are necessary for bactericidal activity. PAA is weakly negatively charged at low pH and adopts a loop conformation in solution. The adsorbed PAA layer is thus relatively thick. In this case, the following adsorbed polycation (DMLPEI) layer is thick and loopy, with many of its positive charges available to interact with bacterial cell membranes. At high pH due to deprotonation, there is an increase in the number of negative charges of PAA which adopts a flat random-coil conformation. The adsorbed PAA layer is flat, and the following polycation layer that is adsorbed interacts tightly. Thus, practically no polycations in brush conformations are generated on the surface. By varying the length of the polycation alkyl chains, they proved the importance of the hydrophobicity of the polycation for its antibacterial activity. The proposed mechanism of action is based on a direct disruption of the bacterial membrane by hydrophobic polycationic chains upon contact with the film.

Polyvinylamines (PVAm) (highly cationically charged polymers) associated with PAA[33] were deposited on cellulosic fibers, reducing bacterial growth by 99.9% after 1 h of contact with at least one layer. Using a quarternized poly(4-vinylpyridine)/carboxymethyl-cellulose (QPVP/CMC) system, Amim et al. showed the importance of the hydrophobicity of the substrate on the antibacterial activity of the resulting PEM.[63] This is due to the chain conformation of the first QPVP layer. In the case of a hydrophilic surface ($Si/SiO_2$ wafer), QPVP chains are thicker (more expanded) due to better hydration, while for a hydrophobic surface (polystyrene films) the chains seem to be less hydrated and tend to be in a more compact conformation. The biocidal activities of QPVP-terminated PEM films built on hydrophobic surfaces were generally weaker than those observed for the ones deposited on hydrophilic ones. Less exposure of pyridinium groups to the aqueous dispersion is obtained in the first case.

To improve their stability under external parameter changes (such as ionic strength or pH changes), PEM films can be either cross-linked after their buildup or treated in a step-by-step manner during each layer deposition.[64] Along these lines, the first reported PEM films that are both antibacterial and antifungal were obtained using carboxymethyl, CHI, and pectin cross-linked by glutaraldehyde.[22] Coated on a polypropylene film, these multilayers were used for tomato packaging and remained almost intact with no apparent rotting infection for 13 days. Yang et al. used click chemistry to assemble, via the layer-by-layer technique, a QAC-containing polymer and a PEG copolymer.[27] The two polymers exhibit antibacterial and antiadherent properties. The final coating exhibited good resistance to bacterial adhesion (97% of reduction) and a high efficiency against marine *Pseudomonas* sp. Moreover, the antibacterial activity of the coating was maintained after exposition to filtered natural seawater at 30 °C for 30 days.

Because PEM buildup is a step-by-step process, multistrata films can be designed, with each stratum having a different property. Therefore, in order to further enhance the properties of antibacterial coatings, in particular during the first 24 h decisive period, a strategy was developed by Wang et al. where an adhesion-resistant PEM film, a thermally cross-linked poly(vinylpyrrolidone) (PVP)/PAA film, was built on top of a contact-killing CHI/HEP film (Figure 7b,c).[65] Incubated in phosphate buffer at pH 7.4 and at 37 °C, the continuous removal of the top PVP/PAA film inhibited *S. aureus* adhesion. After the degradation of the outmost surface, the underlying CHI/HEP film was exposed to the external environment and showed contact-killing properties.

As already mentioned, nanoparticles can be embedded into PEM during the buildup process. This property was used by Van Tassel's group. Single-walled carbon nanotubes (SWNTs) proved to exhibit antimicrobial activity by direct contact, causing severe bacterial membrane damage.[66]

This group dispersed SWNTs in an aqueous solution by using different amphiphile PEG-functionalized phospholipid (PL-PEG) and incorporated them into PLL/PGA films.[59] Compared to pure PLL/PGA films, the antibacterial activity of carbon-based PEMs was enhanced against *E. coli* and *S.*


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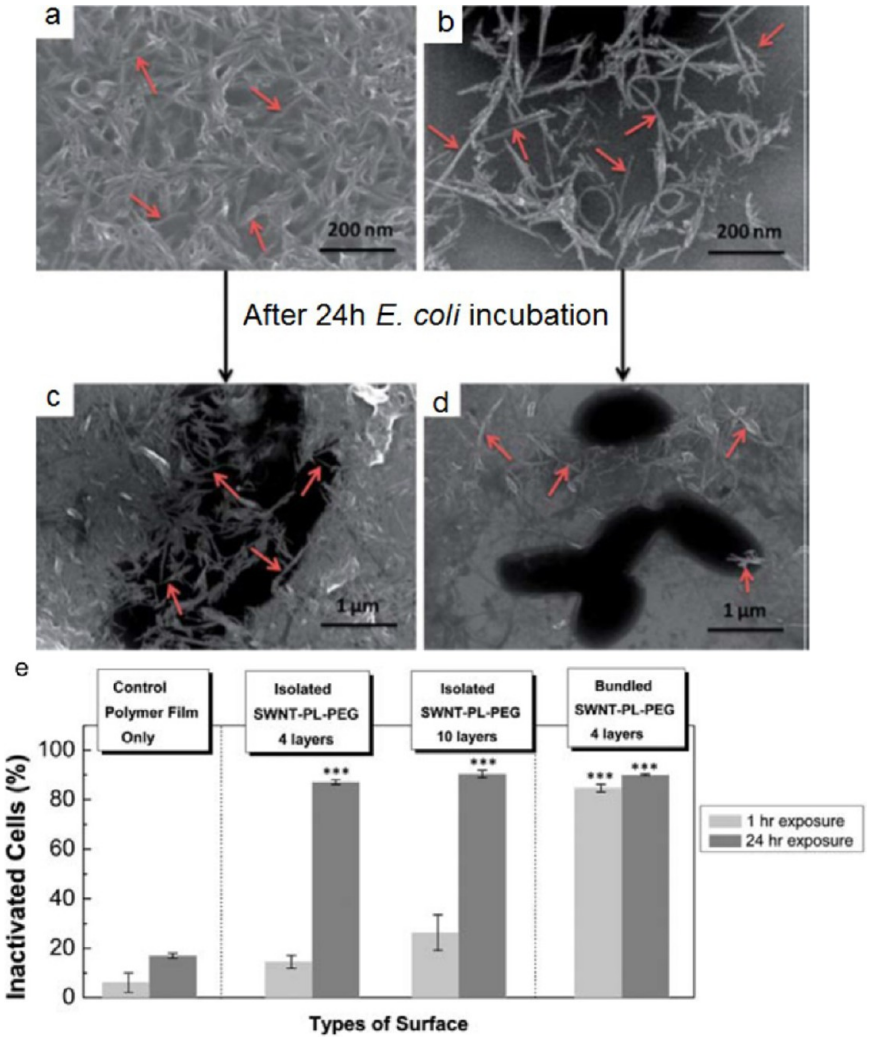


**Figure 8.** Scanning electron microscopy images of (a) (PLL/SWNT-PL-PEGbundled/PGA)$_4$, (b) (PLL/SWNT-PL-PEGisolated/PGA)$_4$, (c) sample (a) following 24 h of *E. coli* incubation, and (d) sample (b) following 24 h of *E. coli* incubation. Red arrows identify some of the SWNTs present. *E. coli* are clearly visible as intact, black objects in (c) and (d). Bacteria appear to be engulfed by the bundled (c) but not isolated (d) SWNT-PL-PEG. (PL-PEG, poly(ethylene glycol)-functionalized phospholipid, PL-PEG). (e) Inactivation of *E. coli* at 1 and 24 h on various substrates, as determined by LIVE/DEAD assay. Reproduced from ref 60 with the permission of The Royal Society of Chemistry.

*epidermidis* at up to 90% inhibition after 24 h of incubation. They also demonstrated the importance of tube bundling effects.[60] Films containing isolated SWNTs inactivated 90% of *E. coli* after 24 h, whereas films containing bundled nanotubes reached this level in only 1 h (Figure 8e). This suggests a fast-acting mechanism possibly related to enhanced SWNT content and/or bacterial contact due to the engulfing of bacteria in SWNT bundles (Figure 8a–d).

PEMs also have the interesting property to allow the loading and the immobilization of enzymes. Sukhishvili's group made use of this property to develop an anti-infective surface by incorporating a biofilm-dispersing enzyme within PEM-coated hydrogels.[61] This can thus be considered to be an active, self-defensive multilayer, a concept that will be further developed later. A biofilm-degrading glycoside hydrolase dispersin B, which cleaves polysaccharides contained in the biofilm matrix, was immobilized in PAH/poly(methacrylic acid) (PMAA) cross-linked PEM films. DispersinB-containing PEMs showed 98% inhibition in biofilm growth in 12 h and good biocompatibility toward osteoblasts. Recently, negatively charged gold nanoparticles and positively charged lysozyme were alternately deposited on negatively charged cellulose mats to obtain an antimicrobial effect with better results against *S. aureus* than against *E. coli*. The weak antimicrobial activity of lysozyme against *E. coli* (Gram-negative bacteria) is due to the protection of the lipopolysaccharide layer surrounding their outermost membrane.[67]

Contact-killing PEM films have a constant efficiency with time, but their action is restricted to the vicinity of the functionalized surface.

## ■ RELEASE-KILLING PEMS

Whereas contact-killing PEM films have an action restricted to their surface, release-killing PEM films contain antimicrobial agents that leach out in their environment. Figure 9 shows the two main strategies that have been used to develop release-killing PEM films: (i) by direct diffusion of antimicrobial agents outside the PEM films[28,30,34,36,44,68−75] and (ii) by degradation of the PEM films and thus the liberation of the antimicrobial agents.[31,76−81] An important parameter to take into account when designing release-killing PEMs is the time scale over which the PEM has to be active. In the case of an implantation,


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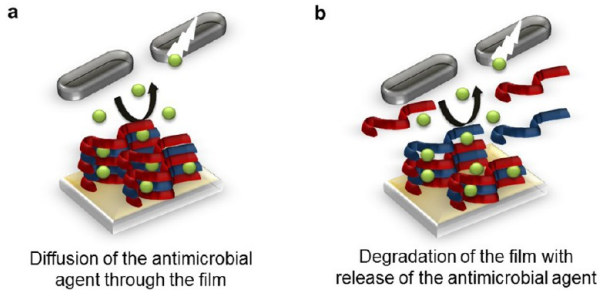


**Figure 9.** Scheme of the different strategies used to obtain release-killing PEMs: (a) diffusion of the antimicrobial agent through the film and (b) release of the antimicrobial agent by degradation of the film.

a 6 h postimplantation period has been identified (decisive period), during which it is of paramount importance to prevent bacterial adhesion and proliferation to ensure the long-term success of the implant.[82,83] This shows that it is usually interesting to target short-time antimicrobial active coatings lasting over 6−12 h up to several days.

The most common antimicrobial agents embedded in PEM films are silver and silver ions ($Ag^+$). The bactericidal properties of silver have been known for centuries. Metallic silver exhibits antibacterial properties when it is dissociated in silver ions.[84] Silver ions act against a broad spectrum of bacterial and fungal strains through binding to the microbial cell membrane and diffusion into the cell. Then $Ag^+$ ions aggregate with proteins or enzymes and inactivate membrane-related enzymes and prevent DNA replication.

Silver nanoparticles (AgNPs) can be directly incorporated into PEM films. For example, PMAA-coated AgNPs could be assembled with PDADMAC in a step-by-step manner on fibers to obtain 80% *S. aureus* growth inhibition for 20 bilayers deposited on silk fibers.[34] To enhance the PEM mechanical properties for implant device functionalization, Kotov's group developed a hybrid PEM film composed of montmorillonite clay nanosheets, PDADMAC, and uncoated AgNPs.[68] To incorporate AgNPs in PEM films, a second strategy based on two steps was used: (i) the buildup of films containing $Ag^+$ ions and (ii) the reduction of $Ag^+$ ions to silver metal. Two methods can be used to obtain $Ag^+$-ion-containing PEM films: loading $Ag^+$ in preassembled PEM films or direct incorporation during PEM buildup. Preassembled PEM films[36,69,70] containing free carboxylic acid or sulfonate groups were immersed in an $Ag^+$ ion solution to trap them in the films by exchange with acid protons. The amount of trapped ions could be modulated by tuning the concentration of free carboxylic acid groups available in the PEM films depending on the pH assembly.[70] Silver ions within the films were then reduced in a second step, in situ, to AgNPs by a chemical reducing agent such as $NaBH_4$[36] or UV radiation.[34,69] After the formation of AgNPs, this procedure regenerated the carboxylic groups of the polyanions within the PEMs, allowing the additional incorporation of $Ag^+$ ions by another immersion of PEM in a $Ag^+$ ion solution.[36] The use of nonphysiological solutions, such as reducing agents, is not suitable for building PEMs directly on biological tissues, including wound beds. To address this limitation, Abbott and co-workers proposed the prefabrication of PEMs loaded with AgNPs on elastomeric stamps and the mechanical transfer of AgNP-loaded PEMs onto the surfaces of biological tissues and biological wound dressings (Biobrane).[36] The mechanical transfer onto soft materials was greatly facilitated by the incorporation of polymeric microspheres (1 to 2 $\mu$m in diameter) into PEMs. The antibacterial activity of Ag-coated Biobrane was tested against *S. aureus* in vitro and in vivo. After 72 h, wounds treated with Ag-coated Biobrane showed significantly ($P < 0.001$) fewer colony-forming units than wounds treated with unmodified Biobrane (more than a 4 log 10 difference).[36] To avoid the addition of any reducing agent for silver ion reduction, the latent reactivity of catechol functional groups within the PEM was exploited.[30] A titanium alloy surface coated with a PEM film composed of CHI/dopamine-modified alginate was simply immersed in an $AgNO_3$ solution to produce and to immobilize AgNPs on the surface.[30] The self-polymerization of dopamine groups was reported to be able to spontaneously reduce noble metal ions to metal nanoparticles, requiring no aid of reducing agents or surfactants and no energy-consuming steps.

In order to even further improve the antibacterial properties of the coatings, Rubner's group designed coatings presenting both a contact-killing ability and containing AgNPs to allow for the continuous release of $Ag^+$ ions. They made use of several potentialities of the step-by-step buildup method to achieve this goal. A multistrata PAA/PAH and PAH/$SiO_2$ nanoparticle film was built and capped with a quaternary ammonium silane. The quaternary ammonium groups conferred to the film strong contact-killing properties. $Ag^+$ ions were then trapped inside the PAA/PAH film by simple immersion in a $Ag^+$ ion solution. By the reduction of $Ag^+$, AgNPs were formed and concentrated at the interface between the PAA/PAH film and the $SiO_2$ nanoparticle layer (Figure 10). This coating showed a killing

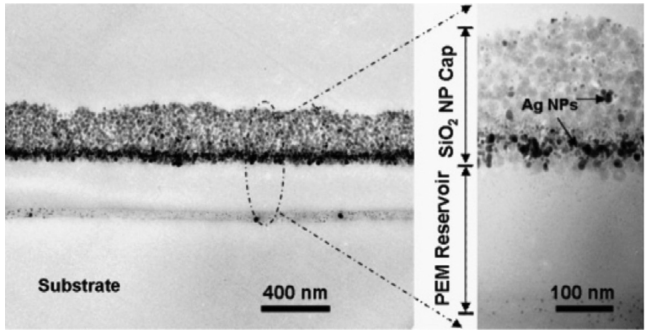


**Figure 10.** Antimicrobial coating presenting both contact-killing properties and $Ag^+$-release-killing properties. The PEM reservoir is constituted of a PAA/PAH multilayer, and the cap layer is constituted of a PAH/$SiO_2$ nanoparticle film where the $SiO_2$ nanoparticles were further functionalized with a quaternary ammonium silane which confers the contact-killing property. The presence of a thin AgNP layer underneath allows the release of $Ag^+$ ions, inducing a release-killing property in the film. Reprinted with permission from ref 85. Copyright 2006 American Chemical Society.

efficiency higher than 99.9% against both strains of bacteria (*E. coli* and *S. epidermidis*) over 4 days.[85] This constitutes a nice example of the great possibilities of the layer-by-layer buildup method in designing sophisticated multifunctional coatings.

Silver ions can also be directly incorporated in PEM films by the alternate deposition of silver ions/polyelectrolyte complexes and oppositely charged polyelectrolytes.[28,71,72] As for the previous strategy, silver ions within the films are then reduced in situ to AgNPs by chemical reducing agents, heating, or UV irradiation. The advantage of this strategy is greater control of the amount of metal ions incorporated into the film, which in turn allows control over AgNP size by changing the


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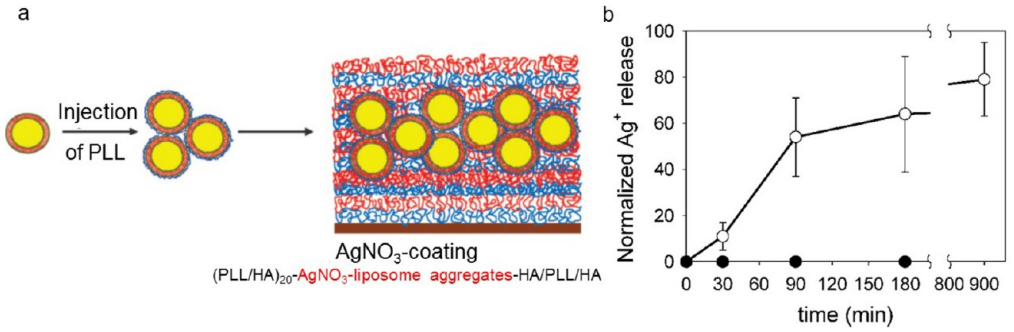


**Figure 11.** (a) Liposome complexation with PLL resulted in the formation of $AgNO_3$–liposome aggregates. They were deposited on a $(PLL/HA)_{20}$ film followed by an additional coating with HA/PLL/HA layers. (b) Release of silver ions from the $AgNO_3$ coating versus time (●) at room temperature and (○) at 37 °C. Reprinted with permission from ref 74. Copyright 2008 American Chemical Society.

concentration of metal ions present during the PEM buildup.[72] Using $SiO_2$ NPs in suspension with a bisamine silver nitrate solution ($[Ag(NH_3)_2]NO_3$) and a PAH solution, AgNP synthesis occurred in situ during LbL assembly, where the amino groups of PAH acted as reducing agents.[72] PAH/$SiO_2$ films were previously reported to be antibacterial coatings, probably due to a combination of their cationic properties and their nanotexturized rough surfaces.[86] To increase the long-term antibacterial performance, Yin and co-workers deposited silanes on a PEI-$Ag^+$/PAA film, creating a superhydrophobic surface that (i) enhanced the stability of the coating, (ii) prevented bacterial adhesion, and (iii) prolonged the release of silver.[73]

Our group introduced an original approach to include silver in a PEM by embedding liposomes filled with $AgNO_3$ (Figure 11a).[74] No silver ions leaked out of the $AgNO_3$-PEM film at room temperature whereas leaking was observed when the temperature was raised to 37 °C (Figure 11b). After 30 min of incubation at 37 °C, 11% of the silver ions encapsulated in the coating were released. This percentage increased to 100% after 15 h. A 4 log reduction in the number of viable *E. coli* was obtained after 2 h of contact at 37 °C. Thus, by raising the temperature to above the transition temperature of the vesicles (~34 °C), the release of silver ions effectively killed *E. coli* populations.

Compounds forming oxygen reactive species known to kill bacteria were also incorporated into PEM films. Polyoxometalates, nanoclusters of transition metals embedded in a CHI-based PEM film, have been used to kill bacteria by the oxidation of their cell membrane.[87] Titanium dioxide ($TiO_2$), known to form biocidal radicals upon UV irradiation, was also used in combination with silver nanoparticles. Yuan et al. built silver-loaded CHI-$TiO_2$/HEP films to obtain a coating with both contact-active properties due to titanium and release-active properties due to silver nanoparticles. In the dark or at low UV-light irradiation, equivalent to the ambient environment in daylight, this coating's antibacterial property is stronger than that of CHI-$TiO_2$/HEP.[88] After 7 days of immersion in PBS, the silver-loaded PEM film maintained its antibacterial activity under low UV-light irradiation. Corbitt et al. built photoactive conjugated polyelectrolyte capsules that can entrap and kill bacteria under exposure to white light (Figure 12).[89] Illumination of the capsules leads to the production of an oxygen singlet at the polymer/bacteria interface, and this oxygen singlet or some subsequently reactive oxygen intermediates interact with the bacteria, resulting in bacterial killing. Figure 12b-c shows that the live-to-dead ratio decreases

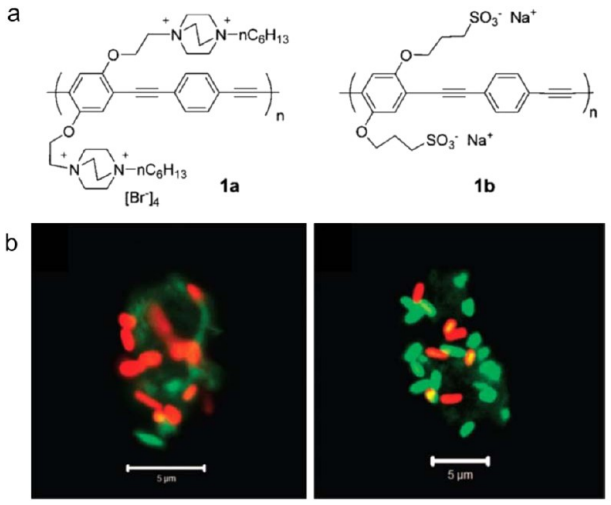


**Figure 12.** (a) Chemical structures of the two charged poly(phenylene ethynylene)-type conjugated polyelectrolytes. Composite confocal microscope images of the hollow CPE capsules with trapped *P. aeruginosa* (b) before and (c) after 15 min of irradiation with the fiber-optic lamp. Live and dead bacteria are labeled in red and green, respectively. Approximate live-to-dead ratios are (b) 7.0 and (c) 0.33. Moderate killing is observed before irradiation by contact with conjugated CPE. Reprinted with permission from ref 89. Copyright 2009 American Chemical Society.

from 7 to 0.33 when hollow polyelectrolyte capsules (CPE) with trapped *P. aeruginosa* are irradiated for 15 min with a fiber-optic lamp. Gabriel et al. reported a photoactive surface coating consisting of cross-linked hyaluronic acid and poly(L-lysine) modified with a photoactive molecule triggered upon irradiation with near-infrared (NIR) light.[90] The strength of such a coating lies in the fact that it can be triggered from outside once capsules are inside the body since NIR light can penetrate tissue. This is not the case for UV light that is used to trigger titanium oxide, for example.

Beside metal ions, the multilayer technology allows also to incorporate antimicrobial organic small molecules into PEMs which can be further released. Antiseptics and antibiotics belong to this category. The chemical structure of different antiseptics and antibiotics that have been embedded into PEMs are represented in Figure 13.

Applied externally to tissues or skin, antiseptics prevent bacteria, fungi, yeasts, and other microorganisms from colonizing a wound. Antiseptics only weaken and slow down the growth of microorganisms, which prevent bacteria from


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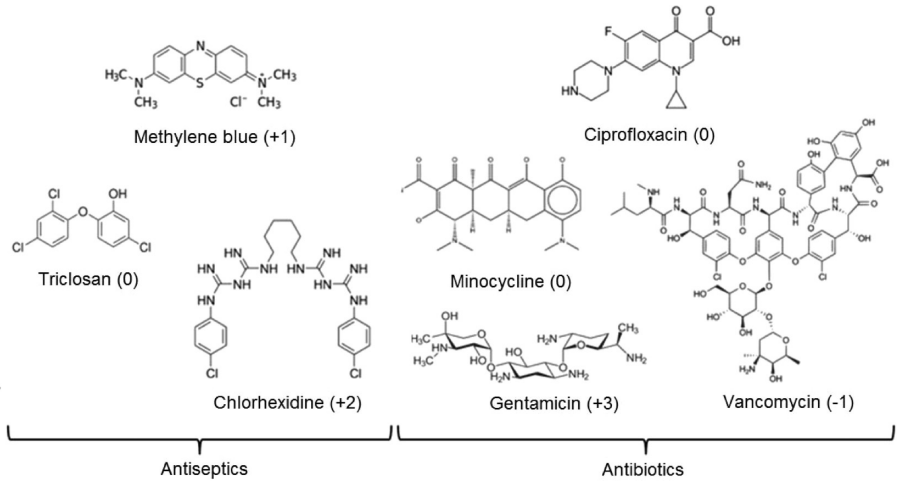


**Figure 13.** Chemical structure, with their respective net charge at physiological pH, of different antiseptics (methylene blue, triclosan, and chlorhexidine) and antibiotics (ciprofloxacin, minocycline, gentamicin, and vancomycin) that have been embedded in PEMs.

causing further infection. Antibiotics are used to kill bacteria by inhibiting the cell wall, nucleic acid, and protein syntheses. They are used to deal with systemic infections. Unlike most antiseptics, bacteria may become resistant to antibiotics after prolonged use.

The insertion of antimicrobial organic molecules into PEMs has been widely studied, and different strategies have been developed. Positively or negatively charged molecules can bind to polyelectrolyte chains. A first strategy to load PEM consisted of a simple immersion of preassembled films into an antibiotic or an antiseptic solution.[20] A second strategy consisted of the direct deposition of molecules either by association with one of the polyelectrolytes or as one of the multilayer components.[31,44,75−78] The loading and release of antibiotics was shown to be tunable by varying the number of deposited layers, the assembly pH, the incubation time, and also by using heat treatment after film buildup.[91] A variety of PEM films have been used to control drug release by diffusion through the films,[44,75] by hydrolytic degradation of one of the polyelectrolytes,[31,76,77] by pH changes,[78] or by external triggers such as the electric potential,[79] laser light,[80,81] and ultrasound.[80,81]

Among the antiseptics used in clinical practice, chlorhexidine (Figure 13) has antimicrobial activity through its positive charge at physiological pH. It destabilizes bacterial cell walls and alters bacterial osmotic equilibrium, leading to the precipitation of the cytoplasmic contents and triggering microbial cell death. Adsorbed alternately with a polyanion, positively charged chlorhexidine was successfully incorporated into PEM films further stamped on wound dressings.[75] Stamped chlorhexidine/PAA PEM films on Biobrane wound dressings led to a localized nontoxic release of chlorhexidine, allowing the decrease in *S. aureus* colonization and promoting normal wound healing in mice.[75] Methylene blue (Figure 13), a cationic dye known for its antimicrobial activity, has been inserted into PEMs as a model bioactive compound, but only Martel's group used its antimicrobial properties.[20] Using the ability of methylene blue to form stable inclusion complexes with $\beta$-cyclodextrin ($\beta$-CD), methylene blue-loaded CHI/poly($\beta$-CD) PEM films were built and showed sustained antibacterial activity against *S. epidermidis* over 72 h of contact.

Due to its hydrophobic nature, it is difficult to incorporate triclosan (Figure 13) directly into LbL-assembled films. Triclosan has a broad antibacterial spectrum and is also an antifungal agent. It is found in numerous consumer products such as soaps or detergents. It is also used for healthcare in surgical scrubs and personnel hand washes. By using dendritic block copolymer micelles, composed of a hydrophobic poly(propylene oxide) core and a positively charged poly(amidoamine) corona, triclosan could be released over a period of several weeks and its activity could be maintained against *S. aureus* growth over this period.[92] Recently, triclosan was encapsulated into cetyltrimethylammonium bromide (CTAB) surfactant micelles that were then inserted by diffusion into a preformed PEI/PAA PEM film.[93] The triclosan-loaded PEI/PAA films could inhibit the growth of both Gram-negative and Gram-positive bacteria by the sustained release of triclosan molecules for over 20 days. On the basis of an exponentially growing PEM film, this coating has the property of healing scratches and restoring its transparency in the presence of water.

Hammond and co-workers developed PEM films based on a hydrolytically degradable poly($\beta$-amino ester) and different polyanionic polysaccharides that efficiently released gentamicin (Figure 13) against *S. aureus* proliferation in vitro and in vivo.[31] Later, vancomycin (Figure 13), a glycopeptide antibiotic, was incorporated into PEMs containing hydrolytically degradable cationic poly($\beta$-amino esters) and anionic polysaccharides. Both the dipping and the spraying methods were used. Whereas films obtained by dipping were exponentially growing, sprayed films grew linearly with the number of deposition steps. The amount of drug incorporated was also very dependent on the buildup method: sprayed films have a 3−8 times higher drug density than the dipped ones. The release time from sprayed films was greatly accelerated compared to the release from dipped films. By combining the dipping and spraying assembly with dextran sulfate and alginate, a composite architecture could be designed that has a rapid initial drug release followed by a linear release above the antibiotic minimum inhibitory concentration.[76] This study provides a new example of the high versatility of the PEM technology to finely tune the coating properties. To prolong the release period of antibiotics embedded in hydrolytically degraded PEM films, one can even add into the architecture inorganic particles such as a laponite clay interlayer that physically block antibiotic diffusion. Using this strategy with two drugs embedded in the same PEM


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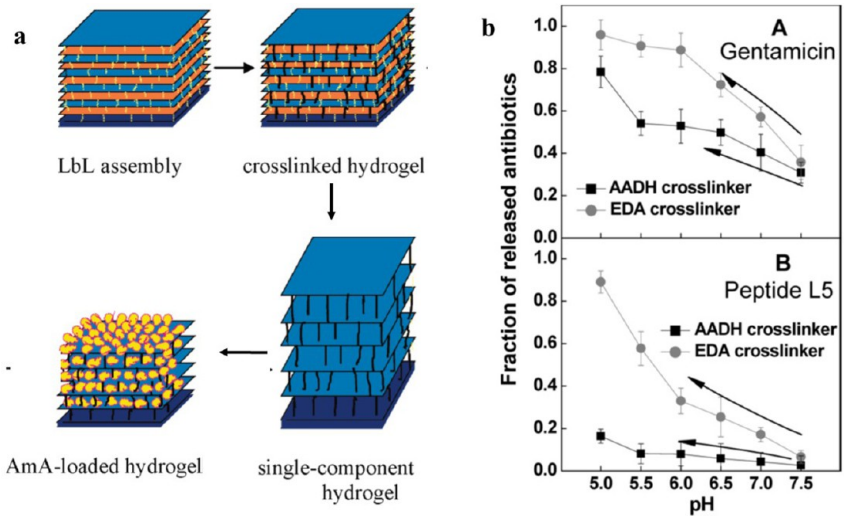


**Figure 14.** (a) Procedure for the preparation of functional antibacterial films. (b) Effect of pH on the retention of peptide L5 from EDA- and AADH-stabilized $(PMAA)_{10}$ hydrogels. The fraction released was determined as the ratio of ellipsometric thickness of peptide loading after pH-triggered release into 0.01 M phosphate buffer containing 0.2 M NaCl thickness to the thickness of antibiotics loaded into $(PMAA)_{10}$ films at pH 7.5. All error bars represent the average standard deviation obtained from three separate experiments. Reprinted with permission from ref 98. Copyright 2010 American Chemical Society.

film, Min et al. obtained a rapid release of gentamicin for the first few days before reaching a sustained release for weeks.[77]

Since tissue injuries, inflammation, and infections commonly lead to a reduced extracellular pH, a pH-triggered release of antibiotics at the implant−tissue interface is very pertinent. Depending on the system, the increase or decrease in pH induces the diffusion of the antibiotics by permeability changes[94] or by a decrease in the binding affinity.[78] Hollow capsules composed of two weak polyelectrolytes PAH and PMAA prepared by LbL assembly showed excellent loading capacity for ciprofloxacin (Figure 13). The pH-induced permeability of microcapsules allowed the loading of ciprofloxacin at low pH and its subsequent release at neutral pH. The release of ciprofloxacin from microcapsules showed significant antibacterial activity against bacterial pathogen *E. coli* for 7 h.[94] While most of the coatings can release antibiotics for a period of some hours to a few days, Zhong and co-workers reached a sustained release over 35 days of minocycline (Figure 13), an antibiotic and anti-inflammatory drug.[78] On the basis of binding-mediated interactions between calcium and minocycline, the antibiotic was incorporated and released from a dextran/gelatin PEM. As the binding affinity decreases with the pH, minocycline was released at pH 6.0 in 13 days with a high initial burst release and at physiological pH in 35 days with a weak burst release.

Recently, several studies have emerged that used an external trigger for the release of antibiotics. Gentamicin (Figure 13) was incorporated into a CHI/Prussian blue PEM film deposited on an electrode to obtain electrically triggered release by the application of a small (<1.0 V) electric potential. When oxidized, the Prussian blue nanoparticles shift from negatively charged to neutral, inducing the dissolution of the film. Different drug release kinetics, i.e., burst, on/off, or pulsatile releases, can be achieved depending on the applied electric potential profiles. The in vitro efficacy of the released drug was confirmed against *S. aureus* bacteria.[79] Combining antimicrobial properties of antibiotics and AgNPs, Raishur and co-workers triggered the release of the antimicrobial agents by laser light or ultrasound due to the rupture of the PEM film.[80,81]

The last family of antimicrobial agents that have been included into release-killing PEMs are AMPs[95−99] and our group was a pioneer in this domain.[95] AMPs, polypeptides secreted by numerous living organisms against pathogens, gained increased attention due to their broad spectrum of antimicrobial activity and their low cytotoxicity. They predominantly act by disrupting the membrane integrity of pathogen agents and thus are unlikely to initiate the development of pathogen resistance. Different strategies were applied to incorporate AMPs in PEM films: either by electrostatic adsorption when they are cationic,[39,95,96,98,100,101] by association with one of the polyelectrolytes when they are poorly water-soluble,[97] or by grafting them on one of the polyelectrolyte components.[29,99] Constituted of 40 amino acids and positively charged, defensin was the first AMP embedded in a PEM film.[95] The antibacterial activity was obtained only for PLL-terminated PEM films. The adhesion of bacteria on the PEM surface was needed for the peptide to be sufficiently in contact with the pathogens, thus exhibiting a contact-killing mechanism. Another positively charged AMP, Ponericin G1, was successfully incorporated into a hydrolytically degradable PEM, based on poly($\beta$-amino ester), to obtain a release killing mechanism.[96] The degradation of the film and thus the release profile depended on the polyanion itself.

Our group introduced antifungal AMP-loaded PEM films and tested them in vivo.[100] Embedded in PLL/PGA PEM films, a small polycationic AMP, chromofungin, was able to interact with the surface of the membrane of the fungi, reducing the growth of *C. albicans* by 64% and fully inhibiting the growth of *Neurospora crassa*. Tested in vivo on rats with an oral candidosis, the chromofungin-loaded PEMs postponed the fungal infection.[100] Using the same PEM system, Karlsson et al. incorporated cationic amphiphilic oligomers of $\beta$-substituted amino acids ($\beta$-peptides) designed to mimic AMPs that exhibited toxicity toward *C. albicans*. $\beta$-peptides were released over a period of 17 days without physical PEM film erosion.[101] Similarly, a cationic $\beta$-peptide-based antifungal agent against *C. albicans* was loaded inside PGA/PLL PEM postfabrication by the incubation of $\beta$-peptides. Then these agents were release


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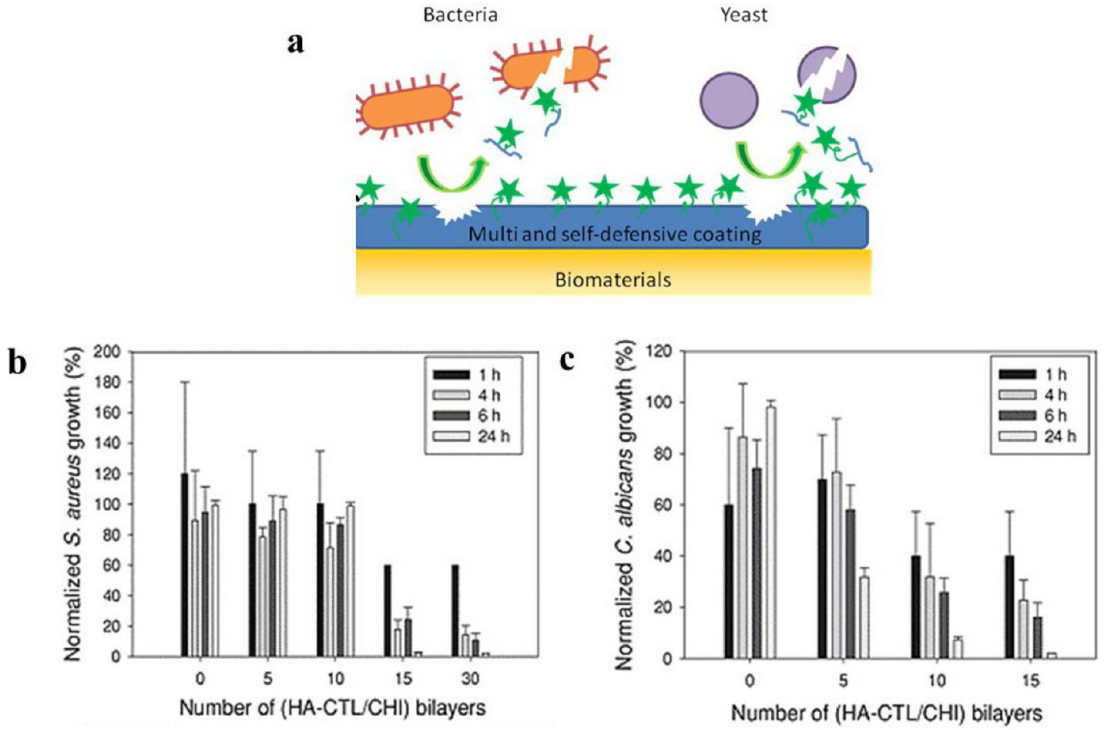


**Figure 15.** (a) Schematic representation of chitosan/hyaluronic acid (CHI/HA) multilayers functionalized by an antimicrobial peptide cateslytin (CTL) and its activity toward bacteria and yeasts based on the enzymatic degradation of the film and the normalized growth of (b) *S. aureus* and (c) *C. albicans* incubated for 1 to 24 h in contact with PEI-$(HA/CHI)_{15-n}$-$(HA\text{-}CTL/CHI)_n$ with $n$ = 0 to 15 and $(HA\text{-}CTL/CHI)_{30}$ multilayer films. Reprinted with permission from ref 99. Copyright (c) 2009 Wiley.

over ~4 months when incubated in physiological media.[37] Using amphiphilic polysaccharides, poorly water soluble AMP (gramicidin A) was incorporated into PEM films.[97] The antibacterial activity of the functionalized PEM films resulted from a double mechanism: contact-killing and release of the peptide into the solution surrounding the film. To functionalize stainless steel, widely used in medical devices, Detrembleur and co-workers developed antimicrobial PEM films based on a water-soluble poly(methacrylamide) bearing oxidized 3,4-dihydroxyphenylalanine groups (Pox(mDOPA)), PAH, and an AMP (nisin).[29] Cross-linking of the PEM films and covalent attachment of nisin were obtained thanks to DOPA moieties at room temperature and without the use of any toxic reagent. Tested against *Bacillus subtilis*, the coating showed sustainable antibacterial activity even after immersion for one night in tap water or after several mechanical cleanings with a wet sponge.

## ■ TOWARD A SELF-DEFENSIVE COATING

Self-defensive coatings can be defined as coatings that become active only in the presence of pathogens. Along this line, Pavlukhina et al. reported the release of antimicrobial agents using pH variations associated with growth of bacteria as an internal releasing trigger. The self-defensive property of the coating is related to a local change in the environment of the coating due to the presence of the pathogens themselves. This was the first system developed on the basis of this idea. Obtained from a preassembled PEM film, Pavlukhina et al. developed an ultrathin PMAA hydrogel, obtained by the layer-by-layer method, able to incorporate and release polycationic charged antimicrobial agents, antibiotics, or AMPs.[98] After the buildup of PVP/PMAA hydrogen-bonded PEM films, PMAA was cross-linked with the use of a cross-linker, ethylenediamine (EDA), or adipic acid dihydrazide (AADH) before the removal of PVP (Figure 14a).

The ultrathin PMAA hydrogel obtained can thus retain its cationic antimicrobial property by an electrostatic mechanism. pH variations related to the growth of *S. epidermidis* were used as an internal trigger to release an antibiotic, gentamicin, and an AMP, peptide L5. In response to this pH variation, the EDA-cross-linked hydrogel swelled while releasing its load (Figure 14b). The antibacterial activity, mainly obtained on the surface of the hydrogel, was demonstrated after 4 h of incubation with Gram-positive bacterium *S. epidermidis*.[98] Sukhishvili's group further developed the concept of a self-defensive coating using the acidification of the immediate environment of pathogenic bacteria. The layer-by-layer assembly of tannic acid (negatively charged natural molecule) and one cationic antibiotic allow the immobilization of a large amount of antibiotics in a stable manner at physiological pH and its release due to the pH decrease.[102] A multilayer nanocomposite, PAA/montmorillonite nanoplatelets, was impregnated by an antibiotic, gentamicin, to further release the antibiotic. PAA-bound gentamicin is released due to the acidification of the environment, whereas gentamicin adsorbed to montmorillonite remained within the coating. Combining both release-killing and contact-killing properties, this coating is also subjected to a swelling that hindered bacterial adhesion.[103] Finally, they developed poly(2-alkylacrylic acid) hydrogels which become hydrophobic and bactericidal in response to bacterially induced acidification of the medium.[104]

Recently our group pushed even further the concept of self-defensive PEM but based on enzymatically degradable PEM films. The self-defensive property was due to the release of AMP triggered only by the hyaluronic acid degradation induced by the pathogens themselves, thanks to hyaluronidase secretion


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(Figure 15a).[99] Cateslytin (CTL), an antibacterial and antifungal peptide, was directly grafted onto HA (HA-CTL) which was then assembled with chitosan. After 24 h of incubation, HA-CTL/CHI films fully inhibit the development of *S. aureus* and *C. albicans* (Figure 15b,c). Furthermore, the coating prevents fibroblast adhesion without inducing cytotoxicity. This highlights a medically relevant application for prevent infections on catheters or tracheal tubes where fibrous tissue encapsulation is undesirable.

## SUMMARY AND PERSPECTIVES IN PEM-BASED ANTIMICROBIAL SURFACES

The first antimicrobial PEM films were reported in the early 2000s, which was only 10 years after the introduction of PEMs by Decher.[1] Since then, the different teams involved in this field aimed at improving the coating properties in order to render them usable for specific application. With rare exceptions, systematic evaluations of the biocompatibility of the antimicrobial PEM coatings were performed in vitro and even sometimes in vivo.[31,75,100] Interestingly and even if complementary studies are needed, it seems that the stiffness of the coating has antagonistic effects on bacteria and cells, i.e., stiffer films promote cell adhesion while they limit bacterial proliferation.[48]

Each strategy to design an antimicrobial PEM film, i.e., adhesion-resistant, contact-killing, and release-killing, was extensively studied. In particular, new polymers were synthesized to bring about better contact-killing properties,[56] and new antimicrobial agents such as antimicrobial peptides were incorporated into release-killing PEM films. Each of the strategies possesses advantages but also drawbacks which can be summarized in Table 2.

**Table 2. Main Advantages and Drawbacks of the PEM Films Built Following an Adhesion-Resistant, Contact-Killing, or Release-Killing Strategy**

| | advantages | drawbacks |
|---|---|---|
| adhesion-resistant | prevent from the first step of biofilm formation, which is crucial | do not kill the bacteria |
| contact-killing | constant efficiency with time | action restricted to the surface |
| release-killing | extensive action | effect limited in time once all of the antimicronbial agent has been released, possible toxicity, possible induction of bacterial resistance |

To further increase the antimicrobial abilities of the coatings, two strategies out of the three main ones were combined, allowing a limitation of the drawbacks. PEM films exhibiting both adhesion-resistant and contact-killing[27,65] or both contact-killing and release-killing properties[85,88] were developed. Release-killing coatings based on the embedding of two different antimicrobial agents were also prepared.[77,88] Some studies were also performed to increase the durability of the coatings. The first aspect was to improve the mechanical properties. This was done by chemically cross-linking the films[29] or by introducing PS microspheres.[36] The second aspect was to obtain long-term release periods, especially by slowing down the diffusion of antimicrobial agents outside the film. For example, a coating with a clay interlayer barrier could slowly release antibiotics for weeks.[77]

An important evolution in PEM coatings was the design of triggered release systems. An internal trigger such as temperature[74] or pH[78,98] or an external trigger such as white light,[89] laser light,[80] near-infrared light,[90] or ultrasound[81] was successfully used. However, the problem of the internal trigger is that the release can be immediate after the pH or the temperature change, and thus the antimicrobial agent can be exhausted before the bacteria are even present. Concerning the external trigger, besides the need for specific equipment, it is not possible to know when the bacteria are present and thus when it is necessary to activate the trigger. One further step was to obtain a smart release that is triggered only by the presence of the pathogen. This was first introduced by Sukhishvili's team using pH changes induced through bacteria proliferation.[98] Our group introduced in 2013 the concept of self-defensive PEM films where the film releases antimicrobial compounds by the enzymatic degradation of the PEM film, which is due to the presence of the bacteria themselves.[99] In contrary to the pH change induced by bacteria,[98] this concept can be extended by using PEM films degradable by only specific enzymes secreted by pathogens. On the contrary to other release-killing strategies, this smart release strategy allows us to maintain the antibacterial property of the coating as long as pathogens are missing.

All of these results suggest that future antimicrobial PEM films will consist of coatings simultaneously presenting several functionalities and also of smart coatings that will become active only in the presence of specific pathogens.

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### Author Contributions

The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

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Lydie Séon studied polymer science at the European Engineering School of Chemistry, Polymers and Materials Science in Strasbourg and obtained her M.Sc. in 2011. She received her Ph.D. in physical chemistry from the University of Strasbourg in 2014. Her research interest was the development of new polyelectrolyte-based coatings exhibiting antimicrobial properties. She is now head of R&D projects at VonRoll Meyzieu (Lyon area, France).



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Philippe Lavalle received his Ph.D. degree in biophysics in 1998 from the University of Strasbourg. After a 2 year postdoctoral position at the Biozentrum in Basel, Switzerland, he was recruited as a researcher at INSERM in 2000 in the biomaterials unit in Strasbourg. He is deputy director of the new INSERM unit "Biomaterials and Bioengineering". The focus of Lavalle's research is the design of mechanical stimuli-responsive materials, smart surface coatings preventing nosocomial infection, immunomodulatory coatings to control monocyte differentiation, and personalized biomaterials.

Pierre Schaaf earned an engineering degree from ESPCI (Paris) in 1982 and a Ph.D. in physical chemistry from the University of Strasbourg in 1986. He was appointed full professor in 1991 at the Chemistry Engineering School of Strasbourg (ECPM). Since January 2013, he has been the director of the new INSERM unit "Biomaterials and Bioengineering". His research interests include polyelectrolyte multilayers and stimuli-responsive and bioactive films.

Fouzia Boulmedais received her Ph.D. in physical chemistry from University Louis Pasteur in Strasbourg (France) in 2003. Her postdoctoral research at ETH-Z in Zürich (Switzerland) and MPI in Gölm (Germany) focused on the electrochemical response of multilayers of polyelectrolytes. She joined the Institut Charles Sadron in Strasbourg (France), where she took a position as CNRS researcher in 2006. Since January 2012, she has been the deputy director of Institut Charles Sadron. Her current research involves polyelectrolyte films: their applications in biomaterials and tissue engineering and their buildup by an electrical stimulus.

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## ■ ABBREVIATIONS

AADH, adipic acid dihydrazide; AgNPs, silver nanoparticles; AMP, antimicrobial peptide; $\beta$-CD, $\beta$-cyclodextrin; Biobrane, biological wound dressing; *C. albicans*, *Candida albicans*; CHI, chitosan; CTL, cateslytin; CTAB, cetyltrimethylammonium bromide; DMLPEI, *N*,*N*-dodecyl,methyl-poly(ethylenimine); *E. coli*, *Escherichia coli*; EDA, ethylenediamine; HA, hyaluronic acid; HA-CTL-C, cateslytin-grafted HA; HA-VB, HA modified by photoreactive vinylbenzyl groups; HEP, heparin; PAA, poly(acrylic acid); PAH, poly(allylamine hydrochloride); PC, phosphorylcholine; PDADMAC, poly(diallyldimethylammonium chloride); PDMS, poly(dimethylsiloxane); PEG, poly(ethylene glycol); PEM, polyelectrolyte multilayer; PGA, poly(L-glutamic acid); PLL, poly(L-lysine); PL-PEG, poly(ethylene glycol)-functionalized phospholipid; PMAA, poly(methacrylic acid); Pox(mDOPA), poly(methacrylamide) bearing oxidized 3,4-dihydroxyphenylalanine groups; PSS, poly(styrenesulfonate); PVP, poly(vinylpyrrolidone); PVPh, poly(4-vinylphenol); QAC, quaternary ammonium compounds; QCHI, quaternized derivatives of CHI; QPVP, quarternized poly(4-vinylpyridine); *S. epidermidis*, *Staphylococcus epidermidis*; *S. aureus*, *Staphylococcus aureus*; SWNTs, single-walled carbon nanotubes; $TiO_2$, titanium dioxide

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