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# Stimuli-Responsive Delivery of Antimicrobial Peptides Using Polyelectrolyte Complexes

*Alexander Antropenko, Frank Caruso, and Paco Fernandez-Trillo**

**Antimicrobial peptides (AMPs) are antibiotics with the potential to address antimicrobial resistance. However, their translation to the clinic is hampered by issues such as off-target toxicity and low stability in biological media. Stimuli-responsive delivery from polyelectrolyte complexes offers a simple avenue to address these limitations, wherein delivery is triggered by changes occurring during microbial infection. The review first provides an overview of pH-responsive delivery, which exploits the intrinsic pH-responsive nature of polyelectrolytes as a mechanism to deliver these antimicrobials. The examples included illustrate the challenges faced when developing these systems, in particular balancing antimicrobial efficacy and stability, and the potential of this approach to prepare switchable surfaces or nanoparticles for intracellular delivery. The review subsequently highlights the use of other stimuli associated with microbial infection, such as the expression of degrading enzymes or changes in temperature. Polyelectrolyte complexes with dual stimuli-response based on pH and temperature are also discussed. Finally, the review presents a summary and an outlook of the challenges and opportunities faced by this field. This review is expected to encourage researchers to develop stimuli-responsive polyelectrolyte complexes that increase the stability of AMPs while providing targeted delivery, and thereby facilitate the translation of these antimicrobials.**

## 1. Introduction

Antimicrobial resistance presents an ongoing growing threat to global health. In 2019, over 3.5 million deaths were associated with antimicrobial resistant strains, with the highest number of cases reported in sub-Saharan Africa.[1] As observed, particularly in non-fermenting Gram-negative pathogens, such as *Escherichia coli* and *Pseudomonas aeruginosa*, the development rate of new antibiotics is superseded by the development of antimicrobial resistance.[2] For example, based on recent data from the World Health Organization, out of the eleven phase 3 antimicrobial agents developed to date, only two have displayed confirmed activity against *P. aeruginosa*.[3] Therefore, alternative strategies need to be explored to address this emergence in antimicrobial resistance.[4–6] One of these strategies is the re-evaluation of antibiotics that have not currently found widespread clinical use.

An example of such a category of antibiotics is AMPs. Although AMPs have excellent antimicrobial properties, their potential has not been fully realized.[7–9] Natural and synthetic AMPs are often positively charged peptides with hydrophobic and hydrophilic moieties, which confer them with antimicrobial properties. These peptides tend to show excellent antimicrobial activity against some of the more challenging Gram-negative pathogens.[10] However, because of their cationic nature and amphiphilicity, they are often toxic to mammalian cells. Moreover, peptides are expensive to manufacture and have short half-lives in biological media owing to degradation by proteases. These limitations make the translation of AMPs into clinical practice challenging, and only a few AMPs have found clinical use, often as last-resort antibiotics.[9,11] Although several strategies have been explored to optimize the pharmacokinetic and pharmacodynamic properties of AMPs, these strategies often

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rely on the chemical modification of the peptides via either using chemical moieties that can temporarily mask the cationic charge[12] or conjugating the AMP to a suitable carrier.[13–16] However, these strategies come at the expense of changing the chemical nature of the AMPs, which inherently changes its antimicrobial profile. Masking the cationic charge will reduce the affinity for negatively charged membranes while modifying the other residues may affect the secondary structure and the hydrophobicity of the AMP. Moreover, the efficiency of these chemical modifications can be hard to control, as AMPs present multiple cationic residues that need masking, leading to variability between batches and manufacturers.[17,18] For example, colistin methanesulfonate, produced by the sulfomethylation of cationic residues in colistin,[19] has different percentages of colistin or methanosulfonated derivatives depending on the manufacturer.[20–22] Since in vitro antibacterial potency is decreased by sulfomethylation, the antimicrobial activity of colistin methanesulfonates is hard to predict.

Alternatively, drug delivery vehicles can protect AMPs from degradation while protecting mammalian cells from AMP toxicity. In this case, the native chemical structure of the AMP is preserved so that, if released, it will keep its original antimicrobial mode of action. The challenge in this approach is to optimize the rate and site of delivery so that therapeutic doses of the AMP are achieved. To date, a wide range of materials have been explored as drug delivery vehicles for AMPs, including liposomes, nanoparticles, and hydrogels.[18,23–31] As most AMPs are cationic molecules, complexation with oppositely charged polyelectrolytes is an attractive and simple method for preparing nanostructured materials from AMPs. For example, layer-by-layer deposition has been employed to immobilize AMPs on surfaces,[32] while complexation in solution can result in the formation of polyelectrolyte complexes and coacervates, including particles and micelles.[33–36] Several factors affect the morphology and stability of the formed polyelectrolyte complexes, including the molecular weight of the polyelectrolytes, their charge density and concentration, or the pH and ionic strength of the solutions used in their preparation. A discussion of these factors is outside this review's scope and has been comprehensively reviewed elsewhere.[32–36] Interested readers are encouraged to check this literature.

Although most of polyelectrolyte complexes can effectively protect AMPs from degradation and shield their toxicity, they provide a passive mechanism of delivery, which can result in low activity, off-target effects, and the development of resistance. However, the presence of pathogenic microorganisms in healthy tissues leads to changes in their microenvironment, including changes in pH, the presence of elevated amounts of specific enzymes secreted by a pathogen, or an increase in the concentration of reactive oxygen species such as hydrogen peroxide. Therefore, the development of stimuli-responsive nanomaterials that can respond to these changes and selectively deliver AMPs at the site of infection is a potential strategy to minimize the limitations of AMPs while addressing the growing concern of antimicrobial resistance.

This review aims to provide an overview of the state-of-the-art in the delivery of AMPs using stimuli-responsive nanomaterials based on polyelectrolyte complexes. In Section 2, we provide examples of AMP delivery systems that use changes in pH as a trigger for delivery as electrostatically assembled nanomaterials are inherently pH-responsive. These systems include pH-responsive delivery from surfaces as well as nanoparticulate systems. We also provide examples highlighting the role of electrostatic interactions between the AMP and the rest of the system on the responsive properties as well as on the delivery of the antimicrobial. In Sections 3 and 4, we provide examples of enzyme- and temperature-responsive materials, respectively. These materials may have a greater potential in realizing targeting against pathogen-mediated stimuli, as infection is associated with the expression of degrading enzymes or changes in temperature. These latter two areas are still in their infancy, with only a handful of examples that use polyelectrolyte complexation to encapsulate AMPs. Thus, we also include examples that demonstrate the potential of targeting these two stimuli to develop smart delivery systems for AMPs that should restrict the release of the AMP to the microenvironment of the pathogen. In Section 5, we conclude with a summary and an outlook of the field. Here, we highlight the key features that make stimuli-responsive polyelectrolyte complexes an ideal platform to deliver AMPs, while also indicating some of the challenges and opportunities for the field. This review is expected to encourage researchers to develop stimuli-responsive PECs that can address the current limitations in the translation of AMPs.

## 2. pH-Responsive AMP Delivery Systems

### 2.1. pH-Responsive Delivery of AMPs from Surfaces

Egles and co-workers were the first to demonstrate that polyelectrolyte complexes could be used to encapsulate AMPs using layer-by-layer deposition. Defensin, an AMP collected from *Anopheles gambiae*, was deposited within polyelectrolyte complex multilayers consisting of polyethyleneimine, poly(sodium 4-styrenesulfonate), poly(allylamine hydrochloride), poly(L-glutamic acid), and poly(L-lysine).[37] In an attempt to prepare multilayered films with antimicrobial properties, defensin was deposited close to the final layer and the polyelectrolyte assembly was capped with either poly(L-lysine) or poly(L-glutamic acid). Antimicrobial activity was observed only for the system that used poly(L-lysine) as the final layer, and this difference was attributed to the better adhesion of the negatively charged bacteria to the cationic groups of poly(L-lysine). Furthermore, at the working pH (6.5–7.0), poly(L-lysine) showed antimicrobial activity owing to the protonation of its amines. A similar approach has been used to develop a variety of systems to deliver AMPs from surfaces but none of the studies to date specifically evaluates the pH-responsive properties of such systems.[38–40]

Sukhishvili and collaborators were the first to report a pH dependence on the release of an AMP using poly(methacrylic acid) as the pH-responsive component.[41] In this work, a model AMP, L5 peptide (containing approximately five positive charges at physiological pH, i.e., 7.4),[42] was loaded onto poly(methacrylic acid) hydrogels through electrostatic interactions between the carboxylate groups of poly(methacrylic acid) and the amines of the L5 peptide (**Figure 1**A). The cross-linker that was used to prepare the hydrogels played a significant role on the pH-responsive release properties of the AMP. When adipic acid dihydrazide (AADH) was used, the hydrogels were weakly pH-sensitive with

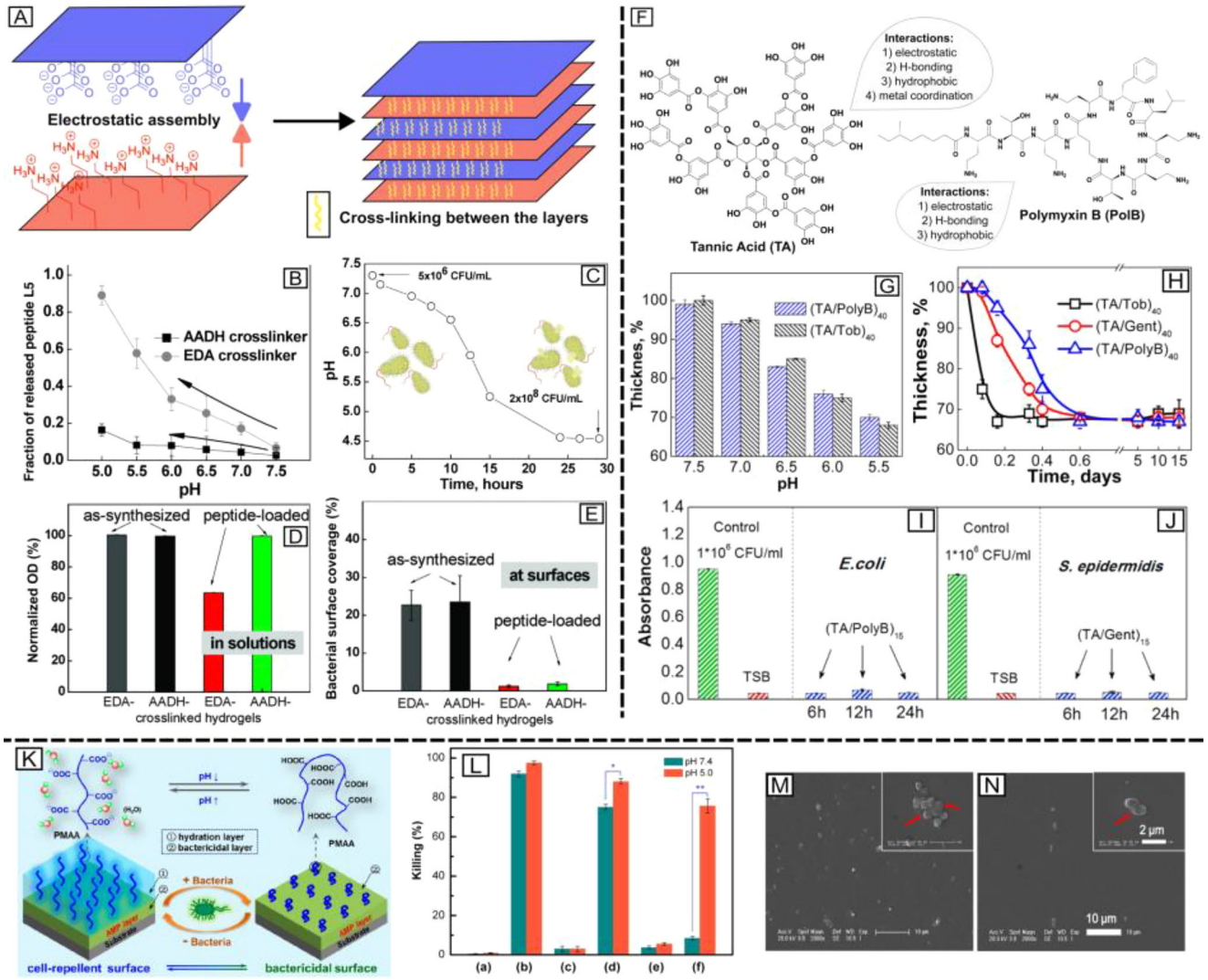



**Figure 1.** A) Schematic representation of polyelectrolyte complex multilayer film formation using oppositely charged electrolytes. B) Effect of pH on the retention of L5 from EDA- and AADH-stabilized poly(methacrylic acid) hydrogels (degree of polymerization = 10). Error bars represent the average standard deviations obtained from three separate experiments. C) Variation in pH during growth of *S. epidermidis* in tryptic soy broth (TSB). D) Normalized optical density at 600 nm ($OD_{600}$) of *S. epidermidis* in TSB after incubation for 4 h in the presence of the as-synthesized or peptide-loaded hydrogels. E) Surface coverage of *S. epidermidis* after incubation for 4 h in TSB. F) Chemical structures of tannic acid (TA) and polymyxin B (PolyB). G) Ellipsometry data of long-term pH-triggered release from $(TA/PolyB)_{40}$ (blue) and (tannic acid/tobramycin)$_{40}$ $(TA/Tob)_{40}$ (black) films, deposited at pH 7.5 and immersed in 0.01 M phosphate buffer solution containing 0.2 M NaCl in the pH range of 7.5–5.5. (H) Time evolution of normalized thickness (as measured by ellipsometry) of (tannic acid/gentamycin)$_{40}$ $(TA/Gen)_{40}$, $(TA/Tob)_{40}$, and $(TA/PolyB)_{40}$ films deposited at pH 7.5 and immersed at pH 5.5. Error bars are within symbol size if not shown. I,J) Growth of *E. coli* (I) and *S. epidermidis* (J) in solution in the presence of $(TA/PolyB)_{15}$- and $(TA/Gen)_{15}$-coated wafers after different incubation times in 2 mL of $1 \times 10^6$ CFU $mL^{-1}$ bacterial suspension at 37 °C; bacterial growth in the presence of uncoated silicon wafers after incubation for 24 h and TSB (controls) is also shown. K) Schematic diagram of bacteria-responsive hierarchical antibacterial surface that can switch from cell-repellent to bactericidal properties upon environment acidification. L) Killing efficiencies of different coating formulations: (a) pristine silicon, (b) AMP, (c) poly(methacrylic acid), (d) one-layer architecture, (e) non-responsive hierarchical architecture, and (f) responsive hierarchical architecture; $^{*}p < 0.05$, $^{**}p < 0.01$. M,N) Representative scanning electron microscopy images of *Staphylococcus aureus* attachment to one-layer architecture (M) or responsive hierarchical architecture (N). Samples were incubated in growth medium containing $10^6$ bacterial cells $mL^{-1}$ for 24 h. Red arrows indicate intact bacterial cells, and red arrows indicate lesions and distortions on the cell membrane of microorganisms. A-E) Adapted with permission.[41] Copyright 2010 American Chemical Society, F-J) Adapted with permission.[43] Copyright 2014 American Chemical Society, K-N) Adapted with permission.[44] Copyright 2016 American Chemical Society.

only 15% of L5 released upon lowering the pH from 7.5 to 5.0 (Figure 1B). This relatively weak pH response was attributed to the strong hydrogen bond interactions between the L5 peptide and the amide groups of AADH. In contrast, when ethylenediamine (EDA) was used as the cross-linker, the hydrogels were more sensitive to changes in pH and released 90% of the loaded L5 across the pH range examined (Figure 1B). Moreover, the majority of the AMP (≈60%) was released between pH 6.0 and 5.0 with an additional 30% released between pH 7.5 and 6.0 (Figure 1B), creating a release profile that favors a low pH.

Further, when compared with hydrogels prepared using cross-linker AADH, the hydrogels that were prepared using EDA as the cross-linker displayed a higher antimicrobial activity in inhibiting growth of *Staphylococcus epidermidis* in media (Figure 1D). This higher antimicrobial activity was likely due to *S. epidermidis* lowering the pH in the culture media once it reached a certain cell density (Figure 1C). However, this effect was mild, with only a 40% reduction in growth of the pathogen observed (Figure 1D). Nevertheless, both AMP delivery systems inhibited colonization of hydrogel-coated surfaces, suggesting that these AMP delivery systems may be more suitable for applications where prolonged delivery is preferred (e.g., antibacterial coatings)(Figure 1E).

In subsequent work, Sukhishvili and co-workers reported an improved antimicrobial delivery system based on tannic acid (TA) (Figure 1F).[43] TA is a naturally occurring polyphenol with multiple binding modalities (i.e., hydrophobic interactions, hydrogen bonding, metal coordination, and/or electrostatic interactions) owing to the presence of the catechol and gallol groups.[45,46] In particular, TA exhibits multiple charges at slightly basic pH (e.g., −6 at pH 9, −3.5 at pH 8.5, and −1 at pH 8)[42] and becomes neutral at most physiological pHs (pH ≤ 7.4) and therefore electrostatic interactions can be easily disrupted. Taking advantage of the unique properties of TA, Sukhishvili and co-workers developed layer-by-layer films that encapsulated either an antimicrobial peptide (polymyxin B, PolyB)[47] or an aminoglycoside antibiotic (tobramycin (Tob)[48] or gentamicin (Gen)[49]), all currently in clinical use as last-resort antibiotics. The TA-derived films displayed a high drug loading capacity (300 μg $mm^{-3}$) and a steady degradation profile with a decrease in pH (Figure 1G,H). Of all three systems, the PolyB-containing films inhibited the growth of *E. coli* after 6 h of incubation in a bacteria-containing solution (Figure 1I) despite having the slowest pH-induced drug release (5% of PolyB released at pH 5.5 after 6 h, Figure 1H). The additional stabilization of the TA/PolyB system was attributed to stronger hydrogen bonding and hydrophobic interactions present in this system than in the other two systems, which was believed to play a key role in the slow-release kinetics observed. The findings of this study illustrate some of the challenges faced when developing an optimized AMP delivery system, in particular the need to balance antimicrobial efficacy and stability of the system under physiological conditions.

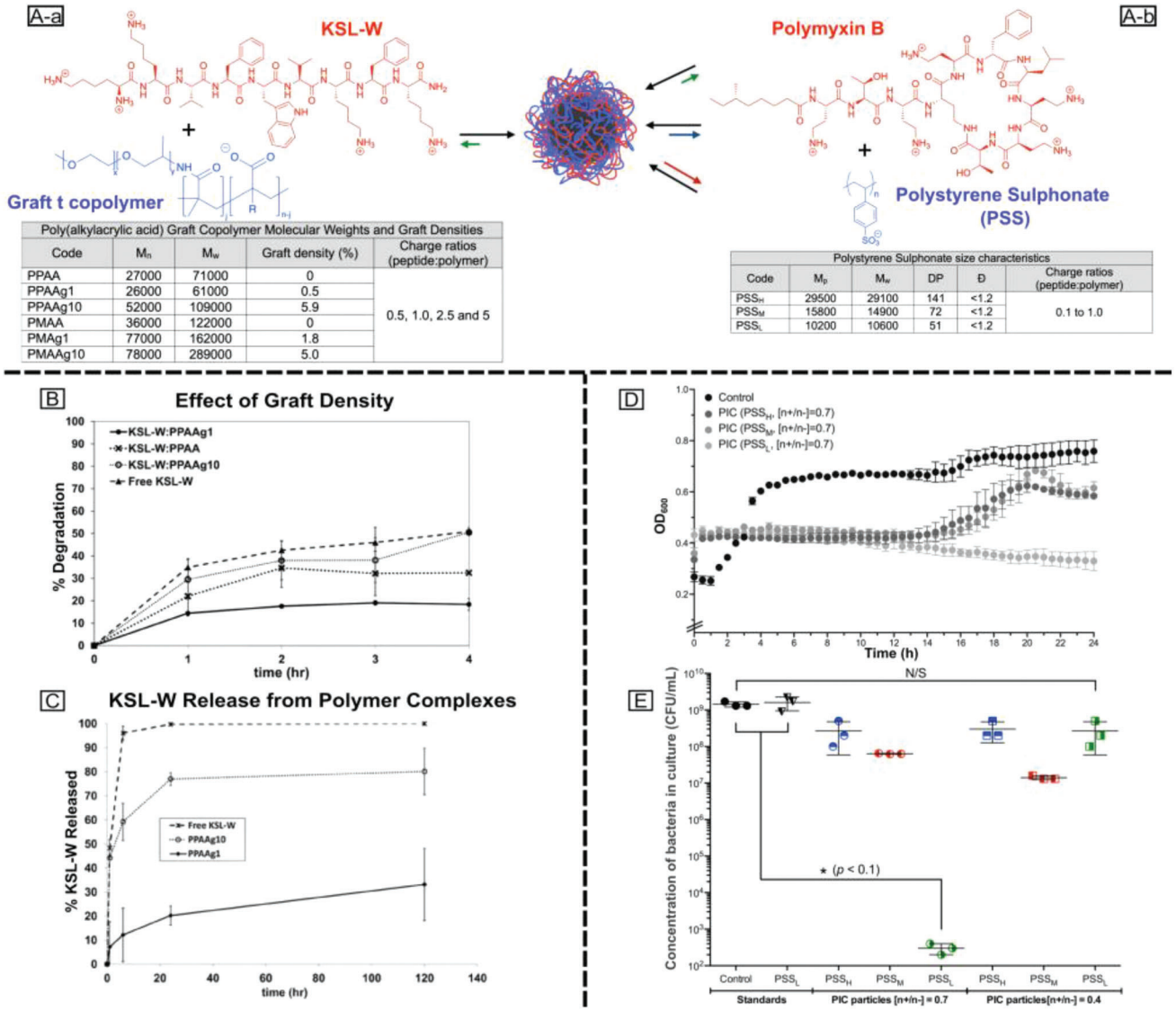
Yin and co-workers developed a pH-responsive surface coating that could switch from displaying cell-repellent properties at physiological pH to bactericidal properties under the acidic conditions caused by bacterial colonization.[44] To realize such switchable properties, a hierarchical polymer brush architecture was used that combined a pH-responsive poly(methacrylic acid) outer layer with an AMP-modified poly(methacrylic acid) inner layer (Figure 1K). Cecropin B, an AMP first isolated from the hemolymph of the giant silk moth *Hyalophora cecropia*, was used as the model antimicrobial.[50,51] Under normal physiological conditions (i.e., pH 7.45), the poly(methacrylic acid) outer layer was negatively charged and hydrophilic, effectively shielding the cationic and hydrophobic AMP, rendering the surface more biocompatible and cell-repellent (Figure 1K). However, with the acidification of the microenvironment caused by bacterial growth, the carboxylate groups of poly(methacrylic acid) became protonated. This protonation rendered the polymer hydrophobic, causing polymer chain collapse and thus exposing the AMP-containing layer (Figure 1K). The study showed that the antimicrobial properties of the polymer brushes containing poly(methacrylic acid) had a high pH sensitivity, resulting in a 65% increase in killing efficacy of *S. aureus* when the pH of the environment changed from 7.45 (15% killing efficiency) to 5.0 (80% killing efficiency) (Figure 1L). In comparison, coatings prepared using a more traditional one-layer approach (i.e., using a copolymer of AMP-modified monomer and methacrylic acid) were only 20% more efficient in bacterial killing at pH 5.0 (90% killing efficiency) than at pH 7.45 (70% killing efficiency) (Figure 1L). As a result, the poly(methacrylic acid)-based polymer brush system showed the best biocompatibility toward blood pellets under physiological conditions (Figure 1M,N). Although this work uses covalent immobilization rather than polyelectrolyte complexation to localize the AMP within the inner layer, this strategy ensures that the cytotoxic nature of AMPs is shielded from healthy tissues when placed in the body, and while at ambient temperature protects the implant from bacterial colonization.

### 2.2. pH-Responsive AMP Delivery from Nanoparticulate Systems

As demonstrated in Section 2.1, delivering AMPs from surfaces may reduce their efficacy in suspension, as a result of the limited diffusion of the antimicrobial from the films. To address this limitation while still protecting these peptides from degradation, systems that can be suspended in biological fluids, such as liposomes or nanoparticles, have been developed.[6,18,27–30] Under the appropriate conditions, polyelectrolyte complexation in solution can produce colloidally stable nanoparticles, often termed PECs, polyionic complexes (PICs), or interpolyelectrolyte complexes. If one of the complexing polyelectrolytes is a block copolymer that carries a neutral block, such as poly(ethylene glycol) (PEG), these nanoaggregates are often termed PIC micelles.[33,52–54]

The first example of an AMP loaded inside a polyelectrolyte complex nanoparticle was reported by Devore and colleagues.[55] KSL-W, a synthetic AMP identified from a combinatorial library,[56] formed colloidally stable particles of 100–200 nm in size with an anionic graft copolymer synthesized via derivatization of poly(acrylic acid) or poly(methacrylic acid) with Jeffamine M-2070 (**Figure 2**A-a).[55] Polymers with different backbones and grafting densities were prepared to control the hydrophilicity and charge density in the polymer backbone. Among the polymers studied, the anionic graft copolymer (PPAAg1), derived from poly(acrylic acid) (PPAA) and with ≈0.5% grafting density (1% grafting density was targeted), performed the best at protecting KSL-W from proteolytic degradation in human plasma, reducing AMP degradation by 30% in 4 h compared to the free peptide (Figure 2B). However, the improved stability caused by shielding was accompanied with a reduction in antimicrobial efficacy of 50% as a result of charge masking, demonstrating the challenge in preparing both active and stable polyelectrolyte complex nanomaterials derived from AMPs (Figure 2C).

In 2017, Fernandez–Trillo and collaborators used a similar approach to encapsulate clinically relevant PolyB with anionic polymer poly(styrene sulfonate) (PSS) (approved by the US Food and Drug Administration) to prepare polyelectrolyte complex particles (Figure 2A-b).[34] Nanoparticles that were stable in physiological media were obtained when an excess of the anionic




Poly(alkylacrylic acid) Graft Copolymer Molecular Weights and Graft Densities

| Code | $M_n$ | $M_w$ | Graft density (%) | Charge ratios (peptide:polymer) |
|---|---|---|---|---|
| PPAA | 27000 | 71000 | 0 | 0.5, 1.0, 2.5 and 5 |
| PPAAg1 | 26000 | 61000 | 0.5 | |
| PPAAg10 | 52000 | 109000 | 5.9 | |
| PMAA | 36000 | 122000 | 0 | |
| PMAg1 | 77000 | 162000 | 1.8 | |
| PMAAg10 | 78000 | 289000 | 5.0 | |

Polystyrene Sulphonate size characteristics

| Code | $M_p$ | $M_w$ | DP | Đ | Charge ratios (peptide:polymer) |
|---|---|---|---|---|---|
| $PSS_H$ | 29500 | 29100 | 141 | <1.2 | 0.1 to 1.0 |
| $PSS_M$ | 15800 | 14900 | 72 | <1.2 | |
| $PSS_L$ | 10200 | 10600 | 51 | <1.2 | |

**Figure 2.** A-a) Schematic representation of the formation of polyelectrolyte complex nanoparticles by self-assembly of cationic KSL-W with anionic graft copolymer (R = H,—$CH_3$, or $CH_2$—$CH_2$—$CH_3$) and effect of the change in multivalency. A-b) Schematic representation of the formation of polyelectrolyte complex nanoparticles by self-assembly of cationic PolyB with PSS and effect of the change in multivalency including size characteristics of polyanions used to assemble the polyelectrolyte complexes. B) Degradation of free KSL-W and KSL-W complexed with anionic copolymer in 50:50 (v:v) human plasma:water. Error bars are standard deviations of 3 samples. C) Release kinetics of free and complexed KSL-W from polyelectrolyte complex nanoparticles made from KSL-W and anionic graft copolymer (PPAAg1—graft density 0.5% and PPAAg10 graft density 5.9%) at 37°C in water (charge ratio = 0.5, $[KSL\text{-}W]_0$ = 66 mg $mL^{-1}$). Error bars are standard deviations of 2 samples. D) Change in $OD_{600}$ for *P. aeruginosa* cultures in the absence (Control) and presence of PSS containing polyelectrolyte complex nanoparticles prepared at {n+/n−} ratio (i.e., ratio between positive charges of ammonium groups in PolyB over the negative charges of acidic groups in PSS) of 0.7. Error bars represent the standard deviation, $n$ = 3. E) Concentration (CFU $mL^{-1}$) of *P. aeruginosa* in the absence (Control) and presence of polyelectrolyte complex nanoparticles prepared from different PSS sources at different {n+/n−} ratios, calculated from the colonies detected on the agar plates (one-way analysis of variance (ANOVA), Tukey test, confidence interval (CI) = 95%); N/S, not significant. Error bars represent the standard deviation, $n$ = 3. A-a, B, C) Adapted with permission.[55] Copyright 2012 Wiley Periodicals Inc., A-b) Adapted with permission under terms of the CC-BY 4.0 license.[34] Copyright 2016, The Authors. Published by Elsevier ltd. D,E) Adapted with permission under terms of the Creative Commons CC-BY 4.0 license.[57] Copyright 2017, The Author(s). Published by Springer Nature.

component was present, resulting in the formation of a "protective" anionic corona around the positively charged AMP therapeutic (Figure 2D).[34] The polyelectrolyte complex nanoparticle loaded with PolyB degraded slowly, providing a passive release of the cationic antimicrobial, and thus inhibiting the growth of *P. aeruginosa* (Figure 2D). Furthermore, by changing the degree of polymerization of PSS, the stability and antimicrobial activity of these polyelectrolyte complex nanoparticles could be fine-tuned (Figure 2A-b,E).[57]

Although the above studies showcase the challenges faced during the preparation of colloidally stable and active polyelectrolyte complexes with AMPs, the effect of pH on the release and

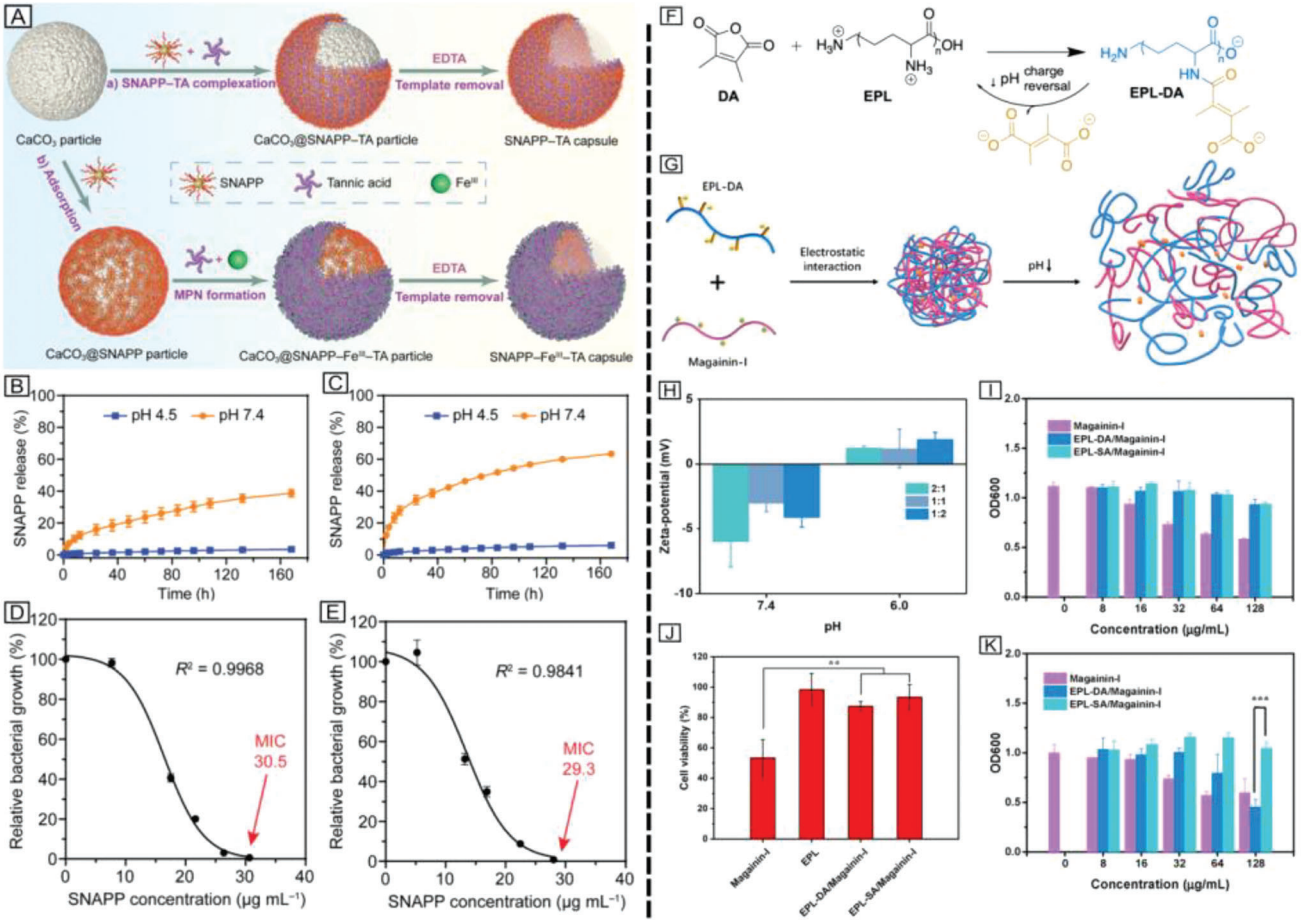



**Figure 3.** A) Schematic representation of the immobilization of SNAPPs via the assembly of SNAPP–TA or SNAPP–$Fe^{III}$–TA capsules onto sacrificial $CaCO_3$ particle templates. B,C) Release of SNAPPs from SNAPP–$Fe^{III}$–TA capsules (B) or SNAPP–TA capsules C) at pH 4.5 and 7.4. The results are shown as means ± standard deviations ($n = 3$). D,E) Relative growth (%) of *E. coli* as a function of the concentration of SNAPPs released from SNAPP–TA (D) or SNAPP–$Fe^{III}$–TA capsules (E). The results are shown as means ± standard deviations ($n = 3$). F) Synthesis of EPL-DA and its pH-triggered degradation. G) Schematic representation of the preparation of polyelectrolyte complex nanoparticles and the stimuli-responsive release of AMP Magainin-I. H) Zeta-potential of EPL-DA/Magainin-I with different compositions at pH 7.4 and 6.0. I,K) Inhibitory effect of Magainin-I, EPL-DA/Magainin-I, and succinic anhydride-modified $\epsilon$-polylysine (EPL-SA)/Magainin-I against *P. aeruginosa* at pH 7.4 (I) and pH 6.0 (K). J) Cytotoxicity of Magainin-I, EPL, EPL-DA/Magainin-I, and EPL-SA/Magainin-I against NIH/3T3 cells. The concentration of Magainin-I was 128 μg $mL^{-1}$. (H,I,J,K) Data were expressed as mean ± standard deviations. A-E) Adapted with permission.[59] Copyright 2021 Willey–VCH, F-K) Adapted with permission.[61] Copyright 2022 Wiley Periodicals LLC.

antimicrobial activity of these systems was not examined. Moreover, in some cases, strong electrolytes were used (e.g., PSS) which are fully charged under most physiological relevant conditions (pH ≤ 7.4).[42] Similarly, most AMPs carry lysine ($pK_a$ of side chain ≈10.7) and arginine ($pK_a$ of side chain ≈12.1) residues, which are fully protonated under these conditions. As such, changes in pH will have a minimal effect on the charge density of these polyelectrolytes, thereby resulting in considerably weak pH-responsive delivery systems.

The first reported pH-responsive polyelectrolyte complex nanoparticles were derived from tannic acid that, much like the system developed by Sukhishvili and coworkers for surfaces,[43] was used to prepare nano systems capable of encapsulating either an AMP—colistin sulphate[19]—or one of two aminoglycoside antibiotics, gentamicin[49] and gatifloxacin.[58] Out of the three antimicrobials tested, the nanoparticles containing colistin sulphate were the most stable at pH 7.4, with only ≈39% of the drug being released at this pH. However, at pH 4.5 the electrostatic interactions between the AMP and the tannic acid were neutralized, leading to complete particle disassembly, and the release of ≈98% of the AMP in < 2 h of incubation. Moreover, the minimum inhibitory concentration of these colistin-containing nanoparticles at pH 7.4 was 50% higher (i.e., weaker antimicrobial activity) than at pH 4.5 and, at this pH, the antimicrobial activity of the polyelectrolyte complex nanoparticles was equivalent to that of free colistin sulphate demonstrating the pH-responsiveness of the system

Tannic acid has also been used by Caruso and collaborators to develop microcapsules that incorporated structurally nanoengineered antimicrobial peptide polymers (SNAPPs),[59] an AMP mimic (**Figure 3**A). SNAPPs are star-shaped poly(amino acid)s containing hydrophilic lysine and hydrophobic valine residues

and exhibit antimicrobial activity (submicromolar range) against multidrug-resistant bacteria.[60] Two types of SNAPP-containing microcapsules were prepared using a template-mediated approach: 1) polyelectrolyte complexation of SNAPPs and tannic acid onto porous $CaCO_3$ (SNAPP–TA capsules) (Figure 3A, route a) and 2) adsorption of SNAPPs on $CaCO_3$ and subsequent encapsulation within a metal–phenolic coating (SNAPP–$Fe^{III}$–TA capsules) (Figure 3A, route b).[59] Both SNAPP-containing systems were more stable at lysosomal pH 4.5 (up to 160 h) than at extracellular pH (i.e., 7.4), at which sustained AMP release was observed (Figure 3B,C), thus inhibiting the growth of model pathogen *E. coli* (Figure 3D,E). The higher stability observed at pH 4.5 was attributed to the formation of stronger H-bonding and ionic interactions between SNAPPs and tannic acid at pH 4.5 when compared to 7.4, possibly due to the star shape architecture of the antimicrobial polymer and the very high charge density of this AMP mimic. Furthermore, the capsules were successfully nebulized into inhalable aerosol droplets, thus demonstrating their potential in pulmonary delivery applications. Compared with the microcapsules formed via route b, the SNAPP–TA capsules (formed via route a) showed higher endosomal/lysosome colocalization, indicating potential to target bacteria that commonly reside within endo/lysosomes (e.g., *Mycobacterium tuberculosis*).

In another study, Ji and co-workers reported a different strategy to prepare pH-responsive polyelectrolyte complex nanoparticles, which exploits the decomposition of 2,3-dimethylmaleic acid amides under acidic conditions.[61,62] A pH-responsive polymer (EPL-DA) was designed by derivatization of $\epsilon$-polylysine (EPL) with 2,3-dimethylmaleic anhydride (DA) (Figure 3F, forward reaction), and the resulting anionic polymer was used to encapsulate a natural AMP, Magainin-I (Figure 3G).[61,63] When the pH of the environment was lowered from 7.4 to 6.0, the charge of the Magainin-I-containing particles changed from negative to positive (Figure 3H), suggesting the hydrolysis of the negatively charged EPL-DA to yield positively charged EPL (Figure 3F, backward reaction). This hydrolysis resulted in charge repulsion between both cationic EPL and Magainin-I, leading to the disassembly of the polyelectrolyte complex particles and consequent release of the AMP. The EPL-DA/Magainin-I nanoparticles displayed minimal antibacterial activity against *P. aeruginosa* at pH 7.45. Notably, at pH 6.0, the EPL-DA/Magainin-I particles retained ≈ 75% of the antimicrobial efficacy of free Magainin-I at concentrations of 32 and 64 μg $mL^{-1}$ while exceeding the efficacy of free AMP at 128 μg $mL^{-1}$ (Figure 3I,K). Furthermore, EPL-DA/Magainin-I particles were considerably less cytotoxic than the free drug—cell viability of EPL-DA/Magainin-I was 87% compared with that (50%) of free Magainin (Figure 3J). These AMP delivery systems were effective at shielding the toxicity of the AMP while triggering its release at low pH.

## 3. Enzyme-Responsive AMP Delivery Systems

### 3.1. Enzyme-Responsive Delivery of AMPs from Surfaces

Although the inherent pH responsive behavior of polyelectrolytes can be exploited to develop AMP delivery systems, challenges remain in preparing stable and active nano systems that can release AMPs as a function of pH, as highlighted in Section 2. Further, as mentioned previously, owing to being strong polyelectrolytes, the charge density of AMPs barely changes within physiological pH. Moreover, changes in pH are not always driven by infection, as there are pH gradients between organs, tissues, and/or cellular compartments. Though these changes can be exploited to develop targeted systems, such as intracellular delivery as shown above,[59] these changes can also lead to off-target effects, such as AMP release in the extracellular milieu. As an alternative approach, changes specifically driven by infections can be used as stimuli. For instance, bacteria often secrete a variety of enzymes, which are virulence factors designed to facilitate colonization of the host and overwhelm its defenses. These enzymes (**Figure 4**A) commonly degrade tissues and biomolecules, often targeting peptide sequences and biopolymers that are not targeted by host enzymes. Therefore, the use of enzyme-responsive antimicrobial delivery systems presents potential to more precisely target and release a variety of antibiotics, including AMPs.[64–66]

Boulmedais and collaborators designed a hyaluronidase-degradable polyelectrolyte complex multilayer coating[67] that incorporated cateslytin (Figure 4B), an AMP that is produced upon proteolysis of chromogranin A, an acidic protein stored in the secretory vesicles of numerous neuroendocrine and immune cells.[68] Hyaluronidase, which is a common enzyme secreted by a variety of pathogens, catalyzes the degradation of hyaluronic acid (HA), a key structural component of the extracellular matrix.[69] As such, hyaluronidases are versatile targets for developing enzyme-responsive delivery systems for a variety of pathogens. Similarly, to the pH-responsive system developed by Egles and co-workers,[37] the AMP was embedded in multilayered films prepared from polyelectrolytes HA and chitosan (CHI). The latter cationic polysaccharide is commonly used in the preparation of polyelectrolyte complex multilayers[70,71] and has antimicrobial properties.[72,73] In this enzyme-responsive system, the AMP was covalently coupled to HA, which resulted in stronger drug retention on the surface. To evaluate the response to hyaluronidase, the antimicrobial activity against *C. albicans* was compared between the HA-based system and a hyaluronidase-resistant system that used polyacrylic acid and cateslytin-functionalized poly(allylamine hydrochloride). The system resistant to hyaluronidase showed minimal antimicrobial effects, whereas the system using HA fully inhibited the growth of *C. albicans* after 24 h of incubation (Figure 4C). Moreover, the versatility of the system was demonstrated by inhibiting the growth of *M. luteus* (Figure 4D) and *S. aureus* (Figure 4E), two hyaluronidase-producing bacteria. Of note, *S. aureus* is a leading cause of pneumonia and other respiratory infections, as well as infections in surgical sites, artificial joints, and cardiovascular tissues.[74]

### 3.2. Enzyme-Responsive Delivery of AMPs from Nanoparticulate Systems

Using similar principles to those discussed above, Fernandez–Trillo and colleagues developed highly selective polyelectrolyte complex nanoparticles by combining a peptide that could be degraded by LasB (a protease secreted by pathogen *P. aeruginosa*) with cationic antimicrobial polymer polyethyleneimine.[75,76] Glutamic acids were incorporated into

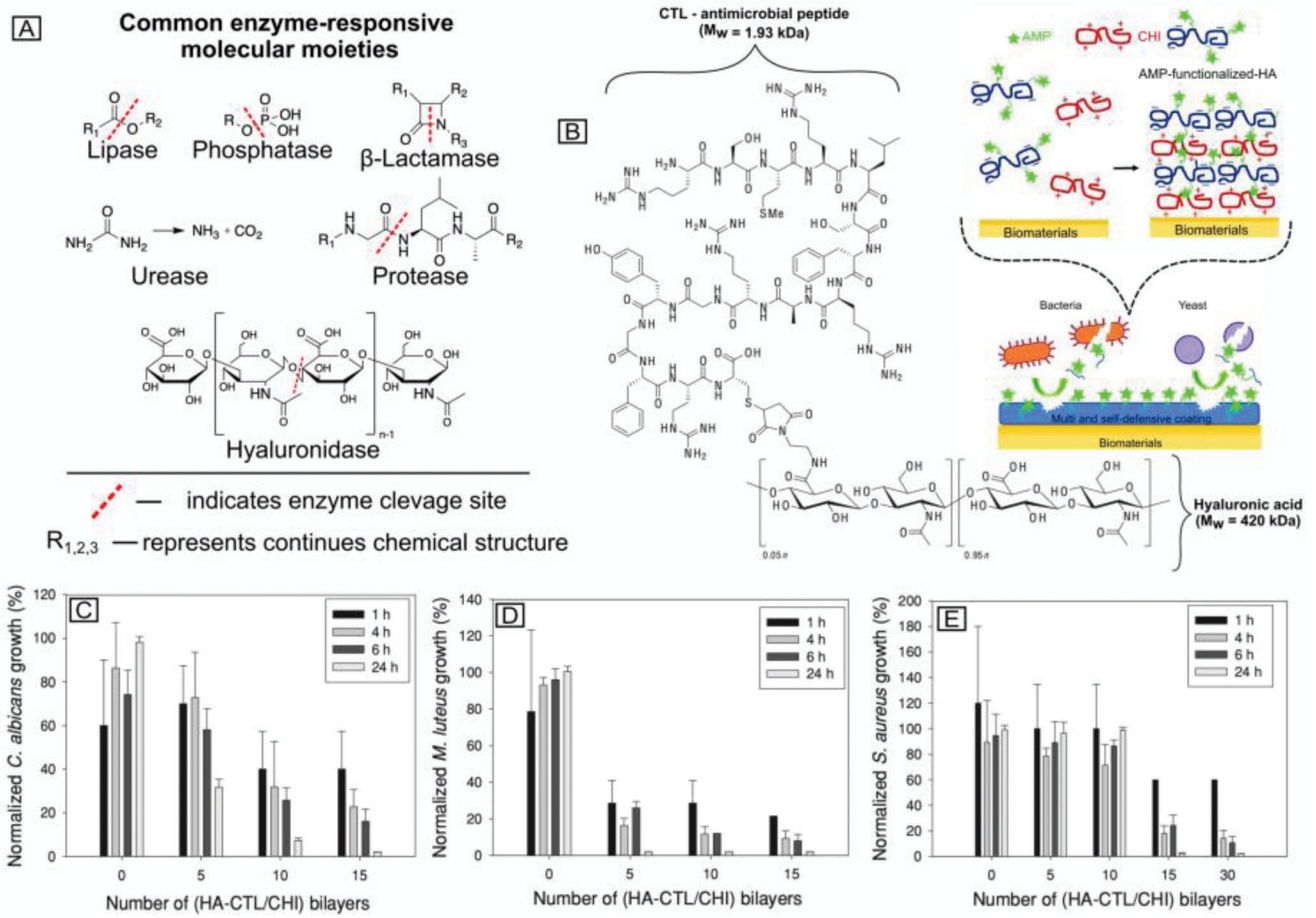



**Figure 4.** A) Schematic representation of enzyme-responsive molecular moieties with their corresponding enzymes used in the antimicrobial drug delivery systems. B) Schematic representation of cateslytin-modified CHI/HA multilayers. C–E) Antimicrobial activity of multi-layered films (HA-CTL/CHI) against *Candida albicans* (C), *Micrococcus luteus* (D), and *S. aureus* (E) after incubation for 1–24 h. B, C, D, E) Adapted with permission.[66] Copyright 2013 Wiley–VCH, A) Adapted with permission under terms of the Creative Commons CC-BY 4.0 license.[67] Copyright 2022, The Authors, published by Wiley–VCH.

the LasB-degradable peptides to facilitate electrostatic interactions with polyethyleneimine, while cysteine residues were incorporated to enable cross-linking within the system via disulfide formation (**Figure 5**A). In this system, the number of anionic and cysteine residues could be easily modified to optimize particle size and stability.[76] These polyelectrolyte complex nanoparticles effectively shielded the cationic charge of polyethyleneimine, significantly reducing its toxicity and antimicrobial activity (Figure 5B, 2 h). Increasing the incubation time of the bacteria with these nanoparticles led to the secretion of increasing amounts of the LasB enzyme, consequently doubling the antimicrobial activity of the enzyme-responsive nanoparticles against *P. aeruginosa* (Figure 5B, 4 h, black bars). Furthermore, the particles displayed excellent selectivity—this increase in antimicrobial activity was not observed when the same experiment was performed with a *P. aeruginosa* strain that could not produce this protease (Figure 5B, 4 h, white bars). Similarly, these nanoparticles were not degraded by human leukocyte elastase (HLE) (Figure 5C, white bars), a protease secreted by our immune system in response to infection.[77] Though this approach faces similar challenges to pH-responsive systems when balancing stability and activity, this work demonstrates the potential of developing fully selective nano systems that release AMPs (and mimics) only in the presence of pathogenic bacteria.

Instead of using oppositely charged polyelectrolytes, Wang and co-workers developed chitosan–antimicrobial peptide (AMP) conjugates (chitosan–peptide conjugates, CPCs) to construct an enzyme-responsive AMP delivery system.[78] These conjugates consisted of three covalently conjugated moieties: a chitosan backbone, an enzyme-cleavable peptide (GPL-GVRGC) coupled to PEG and KLAK,[79] a cationic AMP that forms an amphipathic $\alpha$-helix when bound to negatively charged lipid membranes (Figure 5D). In the absence of gelatinase, these CPCs self-assemble into nanoparticles. However, in the presence of gelatinases, the GPL-GVRGC peptide breaks down, leading to conformational changes that cause the nanoparticles to shift from spherical structures to nanofibers (Figure 5D). As this morphological change occurs, the AMP is exposed, resulting in multivalent electrostatic interactions with bacteria and an antimicrobial effect. As a result of these changes, the minimum inhibition concentration values of the enzyme-degradable system (Figure 5E, red bars) were considerably lower (i.e., stronger

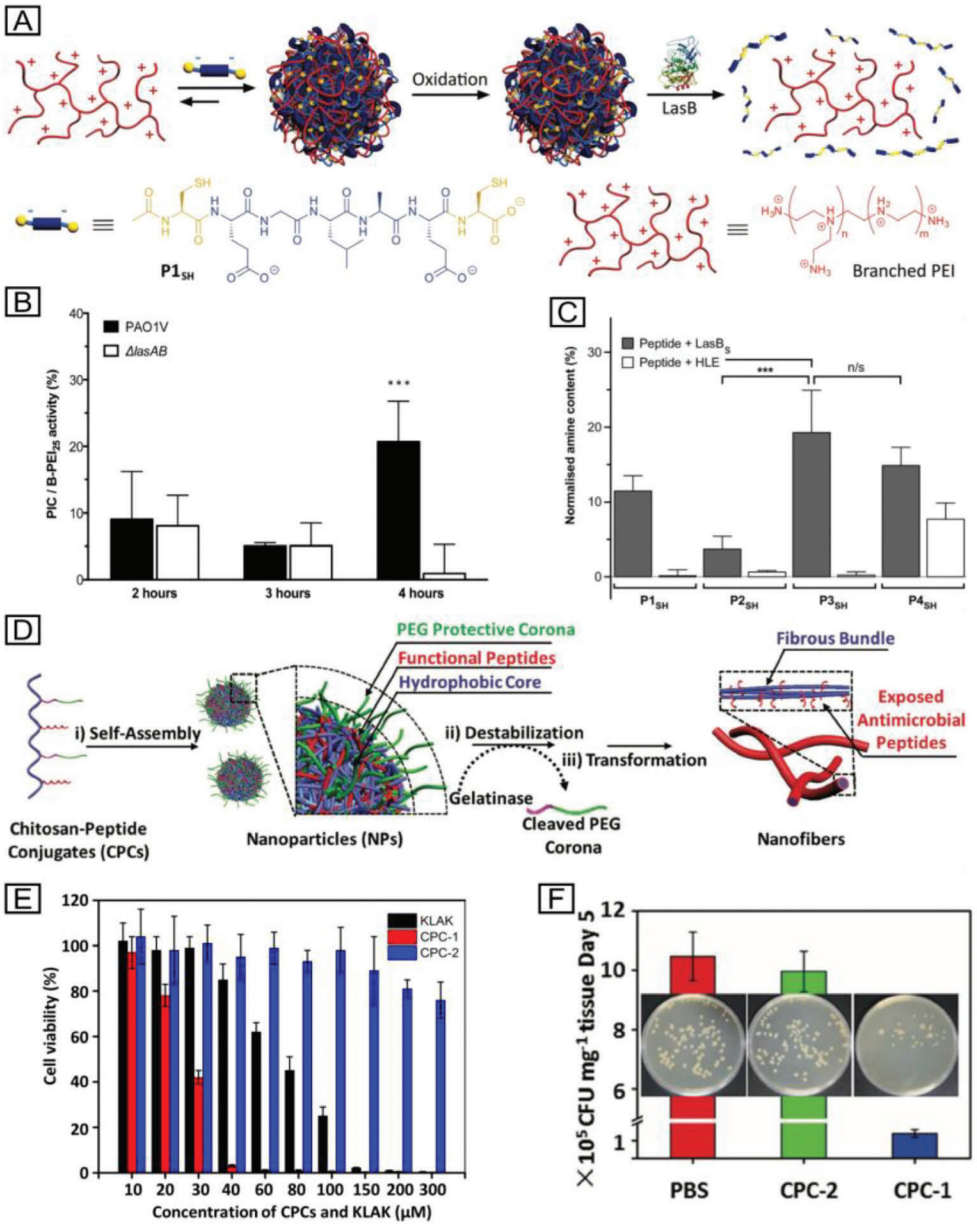



**Figure 5.** A) Assembly and oxidative cross-linking of antimicrobial polyelectrolyte complex nanoparticles constructed from LasB-degradable peptide and antimicrobial polymer branched poly(ethylene imine) (B-PEI). B) Normalized antimicrobial activity as a function of time of polyelectrolyte complex nanoparticles prepared at a N:COOH ratio of 1:0.3. The antimicrobial activity is normalized by dividing the relative antimicrobial activity of the polyelectrolyte complex nanoparticles by that of ($B\text{-}PEI_{25}$) (Mw 25 KDa). $^{***}p < 0.001$ between pathogenic strain of *P. aeruginosa* (PAO1V) and a mutant strain (ΔlasAB) (CI = 99.9%) after 4 h; $n = 3$. C) Relative amine content in LasB-responsive anionic peptides following incubation with LasB or HLE. Data are shown as mean values ± standard deviations ($n = 3$). One-way ANOVA, followed by Tukey's test (CI = 95%) was used to test for significance. n/s, not significant; $^{***}p < 0.001$. D) Illustration of the self-assembly of CPCs and the principle of enzyme-induced morphology transformation. (i) The CPCs self-assemble into nanoparticles with a PEGylated corona. (ii) The protective corona peels off upon cleavage of the peptide in the presence of gelatinase. (iii) Destabilization of the hydrophobic/hydrophilic balance spontaneously promotes reorganization of the self-assembled nanoparticle structures into fibrous structures via chain–chain hydrogen bonding interactions of chitosan. E) Viability of *S. aureus* in the absence and presence of CPC nanoparticles and KLAK (0–300 μM for KLAK). Data are expressed as means ± standard deviations ($n = 3$) and the experiments were repeated at least twice. F) Antibacterial properties evaluated in vivo. The survival of *S. aureus* in infected tissues was quantified on Day 5 by counting the number of colonies in infected tissues from mice injected with phosphate-buffered saline (PBS), enzyme-responsive (CPC-1), or non-responsive (CPC-2) nanoparticles. Data are shown as mean values ± standard deviations ($n = 5$). A, B) Adapted with permission.[75] Copyright 2016 The Royal Society of Chemistry, D-F) Adapted with permission.[78] Copyright 2017 Wiley–VCH, C) Adapted with permission under terms of the Creative Commons CC-BY 4.0 license.[76] Copyright 2018 The Authors, published by Wiley–VCH.

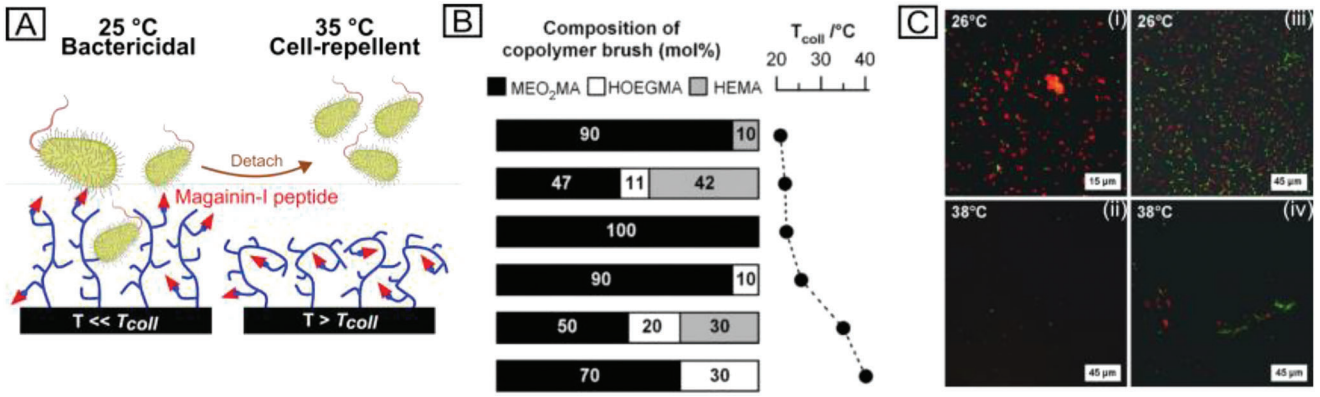



**Figure 6.** A) Schematic representation of the brush conformation below and above LCST of copolymer brushes derived from 2-(2-methoxyethoxy)ethyl and oligoethyl methacrylate. B) Collapse transition temperature ($T_{coll}$) of copolymer brushes depending on the composition of the monomer and (2-(2-methoxyethoxy)ethyl methacrylate, $MEO_2MA$; oligoethyl methacrylate, HOEGMA; hydroxyethyl methacrylate, HEMA). C) Colonization by *L. ivanovii* (i,ii) or *E. coli* (iii,iv) of surfaces coated with polymer brushes. Samples were incubated at 26 or 38°C. A-C) Adapted with permission.[86] Copyright 2010 Wiley–VCH.

antimicrobial effect) toward gelatinase positive *S. aureus* compared to the nondegradable system (Figure 5E, blue bars) and to the AMP (Figure 5E, black bars). Furthermore, in vivo studies using a mouse model confirmed the high antimicrobial efficacy of the enzyme-responsive particles (Figure 5F, blue bar), as shown by a 10× decrease in *S. aureus* colony counts compared to the nonresponsive control (Figure 5F, green bar) and to the negative control (Figure 5F, red bar).

## 4. Thermoresponsive AMP Delivery Systems

Although enzyme-responsive delivery of AMPs has the potential to be very specific, it may be limited to applications where the pathogenic agent is known. This strategy is often required in the treatment of resistant infections that do not respond to broad-spectrum antibiotics. Conversely, developing strategies that can target a broader range of bacteria has advantages as a first line of defense against infections. The presence of a pathogen within the human body induces an immune response that commonly leads to inflammation and an increase in tissue temperature.[80–82] These changes in temperature can be localized (e.g., wounds) or systematic (e.g., fever). Local heating of a tissue can also be achieved externally (e.g., using microwave irradiation, infrared illumination, and ultrasound irradiation), which has been used extensively for the thermoresponsive release of anticancer drugs.[83–85] To date, AMPs have been used primarily in thermoresponsive coatings and topical applications, wherein the difference between the internal temperature of the body (i.e., 37°C) and the external environment is used as the main stimuli.[66,86–89]

One of the most common thermoresponsive materials used for antimicrobial delivery is poly(*N*-isopropylacrylamide).[90] This thermoresponsive polymer has a low critical solution temperature (LCST) of 32°C, which is close to the typical human body temperature of 37°C.[90,91] Below the LCST (e.g., room temperature), poly(*N*-isopropylacrylamide) chains are hydrophilic, as the amide groups form hydrogen bonds with water that help solvate the polymer. Above the LCST (e.g., body temperature), hydrogen bonds with the solvent weaken, leading to the collapse of the poly(*N*-isopropylacrylamide) chains in aqueous solutions, which become hydrophobic and potentially precipitate out of solution. Therefore, switchable antimicrobial systems can be produced with drugs attached to the hydrophilic or hydrophobic moieties of the poly(*N*-isopropylacrylamide) polymer.[66,92] Although poly(*N*-isopropylacrylamide) has been used in various biomedical applications,[93,94] some indications of polymer cytotoxicity have been reported under certain conditions.[87]

Therefore, alternative polymer matrices with thermoresponsive properties are also being considered such as copolymer brushes derived from 2-(2-methoxyethoxy)ethyl and oligoethyl methacrylate, as developed by Glinel and collaborators (**Figure 6**A).[86] The transition temperature of the polymer brushes could be fine-tuned by adjusting the content of oligoethyl methacrylate and the length of the oligoethyl methacrylate side chains, or by introducing a more hydrophilic monomer such as hydroxyethyl methacrylate,[92,95] to achieve LCSTs similar to that of poly(*N*-isopropylacrylamide) (Figure 6B). To obtain antimicrobial properties, Glinel and co-workers grafted the AMP magainin-I[63] onto the oligoethyl methacrylate moieties of a brush copolymer that had a transition temperature of 35°C (Figure 6B). These polymer brushes were grown from silicon wafers to create surfaces with thermoresponsive antimicrobial activity.[86] In a similar fashion to the pH-responsive coating developed by Yin and coworkers,[44] the AMP was exposed to an environment below the LCST of the brush copolymer, successfully inhibiting the growth of pathogens *E. coli* and *Listeria ivanovii* (Figure 6C). However, at temperatures above the LCST of the brush copolymer, progressive collapse of the brushes occurred, which buried magainin-I within the polymer network and concurrently exposed the hydrophobic moieties of the brush copolymer, making the surface repellent to these bacteria. These polymer brushes represent the first example of antimicrobial coatings for biomedical devices that are bactericidal at room temperature (i.e., below the LCST) while inactive and cell-repellent under physiological conditions (i.e., above the LCST).

In the example presented above, the AMP was covalently immobilized onto the thermoresponsive polymers, which helped with drug retention on the surface and increased the lifetime of the antimicrobial coatings. Moreover, this covalent

attachment was critical to the mode of action of the antimicrobial coatings in preventing leaching from the surface and ensuring that the AMP would be shielded above the LCST. However, for systemic delivery and topical applications, self-assembled thermoresponsive polyelectrolyte complexes loaded with AMPs are expected to be better suited and warrant further investigations. To date, there are no examples of thermoresponsive polyelectrolyte complexes loaded with AMPs, although this strategy has been explored in other therapeutic areas.[84,96,97] For instance, Möller and colleagues have developed promising thermoresponsive polyelectrolyte complex nanoparticles consisting of anionic poly(*N*-isopropylacrylamide-*co*-acrylic acid) and cationic EDA-modified cellulose, which were loaded with an anionic model drug zoledronate,[98] which is used against osteoporosis.[99] The LCST of the polyelectrolyte complex nanoparticles was considerably higher (41–48°C) than that of pure poly(*N*-isopropylacrylamide) solutions (33°C) possibly due to the introduction of hydrophilic moieties (e.g., acrylic acid) in the system.[100,101] Therefore, the heat-dependent release of zoledronate was shifted toward higher temperatures, with only 40% of the drug released (within 24 h) at physiologically relevant temperature of 37°C. The release was thus gradual and sustained, starting from a low temperature of 25°C and progressing toward higher temperatures. This release profile was unexpected given that free poly(*N*-isopropylacrylamide) is known for its sharp phase-transition. However, such gradual release kinetics would be beneficial when a sustained delivery of therapeutic doses of AMP for a prolonged period of time is required.

The poly(*N*-isopropylacrylamide-*co*-acrylic acid)/EDA-modified cellulose polyelectrolyte complex also showed pH-responsive behavior, which resulted in significant changes in particle size. At pH 7.0, the particles were small and monodisperse, owing to the deprotonation of the acrylic acid moieties that strengthened the electrostatic interactions. In contrast, at pH 4, the particles became larger, thereby destabilizing the electrostatic interactions, which consequently facilitated drug release. This work, while not focused on the release of AMPs, exemplifies the potential of delivery systems based on polyelectrolyte complexes to exhibit multiple responses (in this case pH and temperature). This capacity to respond to multiple stimuli can potentially be used for more effective drug release at the site of infection, in response to both inflammation and pH acidification of the bacterial microenvironment.

## 5. Conclusion

We have provided a critical overview of the delivery of AMPs using stimuli-responsive polyelectrolyte complexes. These systems are ideally placed to producing new delivery systems for this family of antimicrobials in further unlocking their potential. The key features that make polyelectrolyte complexes particularly suitable for the stimuli-responsive delivery of AMPs are as follows:

- Most AMPs are cationic and can thus be encapsulated within polyelectrolyte complexes without requiring their chemical modification (e.g., via covalent conjugation). Preserving the chemical structure of the AMP should minimize any risks of compromising their mode of action and activity.
- Polyelectrolyte complexes are inherently sensitive to changes in pH and therefore can be used to target bacteria that affect the pH in their microenvironment. Moreover, this pH response can be used to target intracellular bacteria and promote the delivery of AMPs within cellular compartments such as the endosome.

The first part of the review has provided key examples that demonstrate the potential of polyelectrolyte complexes to deliver AMPs in response to changes in pH. Challenges faced in the development of these materials are discussed, in particular how to balance activity and stability. Furthermore, the highlighted examples demonstrate the ability of these systems to deliver a range of AMPs, including some that are in clinical use such as PolyB[34,57] and colistin sulfate.[102] Delivery from both surfaces and nanoparticulate systems have been developed, and these systems have been used to target a range of bacteria including some of the most virulent and antibiotic-resistant pathogens such as *S. aureus*[102] and *P. aeruginosa*.[75,76] The release of the AMP in many of these systems is triggered at low pH, as the anionic polyelectrolytes are neutralized at that pH, which results in weakened electrostatic interactions, which hold the polyelectrolyte complexes together. These materials may therefore be suitable for intracellular delivery of AMPs, targeting pathogens that commonly traffic through the endosomes such as *M. tuberculosis* and *Listeria monocytogenes*. A noteworthy class of systems is one that can switch their charge in response to pH such as the nanoparticles developed by Ji and co-workers (Figure 3F,G).[61,62] The ability to reverse the charge should not only accelerate the release of the AMP, due to charge repulsion, but also provide additional antimicrobial properties by forming cationic polymers.

Bacterial infection is often accompanied by changes in parameters other than pH, which can also be exploited as alternative stimuli triggers. These changes include an imbalance in the concentration of enzymes [103,104] or increases in temperature.[80–82] The rest of the review showcases examples of AMP delivery in response to other stimuli, namely enzymes and changes in temperature. Enzyme-responsive delivery of AMPs from polyelectrolyte complexes is still in its infancy, with only a handful of examples reported in the literature. However, these examples highlight the potential of this strategy to develop systems that can target common enzymes[103,104] that are expressed by a range of pathogens, such as the hyaluronidase-responsive surfaces developed by Boulmedais and collaborators (Figure 4B),[67] and as such target a range of pathogens including bacteria and fungi. Alternatively, enzyme-responsive delivery can be used to target a single pathogenic species due to the specificity of the polyelectrolytes used (Figure 5A).[75,76] This strategy may be ideal to treat resistant infections that do not respond to broad-spectrum antibiotics. Once the pathogenic agent is known, enzyme-responsive systems should restrict the release of the AMP to the microenvironment of the pathogen, minimizing the systemic delivery of the antimicrobial and thus, off-target effects including toxicity and the development of resistance in commensal bacteria. With regard to using temperature[80–82] as a stimuli, there are currently no examples of specifically using thermoresponsive polyelectrolyte complexes to deliver AMPs. However, future studies are warranted given that infection often leads to local inflammation, and the associated increase in temperature[80–82] provides a

localized stimuli to trigger AMP release. This review, therefore, includes a couple of examples that highlight some of the best features that this approach can offer. For example, temperature can be used to prepare switchable surfaces that, for instance, are bactericidal at room temperature while not active and cell-repellent under physiological conditions. This principle has been exemplified by Glinel and collaborators (Figure 6A) using an AMP covalently attached to a thermoresponsive polymer[86] but is yet to be demonstrated for the development of polyelectrolyte complexes that incorporate AMPs. However, thermoresponsive polyelectrolyte complexes have been used to trigger the release of other therapeutic agents in response to increases in temperature. As the LCST of the polymers and polyelectrolytes used can be fine-tuned, we can anticipate the development of thermoresponsive polyelectrolyte complexes that trigger the release of AMPs > 37–38°C to respond to the increase in temperature[80–82] associated with infections. Moreover, as polyelectrolytes complexes are intrinsically pH-responsive, the use of polyelectrolytes complexes in realizing dual-responsive delivery offers a viable avenue when compared to systems non-based on polyelectrolytes complexes, in achieving antimicrobial systems that can target the multifaceted aspects of microbial infection. Finally, although not included in this review, endogenous stimuli such as light or ultrasound could also be used to trigger the release of AMPs from polyelectrolyte complexes. These stimuli have been exploited to deliver other therapeutic agents, and they could be ideally suited to develop topical applications of AMPs where the location and penetration of the stimuli can be precisely controlled.[105–110]

As mentioned previously, the main challenge when developing this type of delivery system is to balance the stability of the polyelectrolyte complexes with their antimicrobial activity. In a laboratory setting, the antimicrobial activity of the polyelectrolyte complexes is often smaller than that of the native AMP, as some of the antimicrobial remains encapsulated within the delivery vehicle. For conventional polyelectrolyte complexes maximizing stability will come at the expense of compromising activity. This limitation may be resolved during drug and clinical development by optimizing the concentration and dosing regime of the polyelectrolyte complex formulation so that therapeutic doses of the AMP can be reached without triggering any unwanted effects. Additionally, stimuli-responsive systems have the potential to address this limitation by reaching similar activities to those of the native AMP as long as they can accelerate particle decomposition once a stimulus is applied. Degradable polyelectrolytes should be particularly suited to achieve this acceleration, with enzyme-responsive materials the most likely candidates. However, as observed for some of the examples reported in Section 3, the activity of the enzyme-responsive system can remain below that of the native AMP. To accelerate degradation, some of these systems may need to be coupled with self-immolative materials with high decomposition rates due to a domino effect that triggers a self-programmed degradation in response to external stimuli. Self-immolative materials that respond to pH and enzymes have already been reported in the literature, and their application to develop stimuli-responsive polyelectrolyte complexes inspired by those reported above could address the current limitations observed when balancing stability and activity.[111,112]

From a clinical perspective, developing stimuli-responsive polyelectrolyte complexes that can selectively deliver therapeutic doses of AMPs at the site of infection has the potential to circumvent the off-target toxicity of many of these antimicrobials. This toxicity often prevents their translation to clinical use. As outlined above, the main challenge would be balancing antimicrobial efficacy and stability. Still, once this challenge is resolved, researchers would also have to consider overcoming the regulatory requirements needed to deliver a clinically viable product. As such, the best approach could be to focus on AMPs already approved for clinical use. For example, PolyB is approved for topical use to treat minor cuts, scrapes, or burns. As such, regulatory approval of a stimuli-responsive polyelectrolyte complex that delivers PolyB topically would focus on approving the new delivery vehicle, simplifying the translational pathway. This development may then set precedence and give a competitive advantage to develop a family of products that can deliver other non-approved AMPs with unique modes of action and antimicrobial activities.

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## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

antimicrobial peptides, drug delivery, polyelectrolyte complexes, stimuli-responsive materials

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