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"Just Antimicrobial is not Enough" Revisited—From Antimicrobial Polymers to Microstructured Dual-Functional Surfaces, Self-Regenerating Polymer Surfaces, and Polymer Materials with Switchable Bioactivity

*Maria Zober and Karen Lienkamp**

Biofilm formation can be slowed down by restricting protein adhesion on a surface, or by antimicrobial/biocidal activity of the material (among other methods). In this progress report, the recent work on alternatives to single component antimicrobial or protein-repellent polymer materials is presented. These are microstructured bifunctional polymer surfaces and self-regenerating polymer multilayer stacks. The microstructured polymer surfaces consist of antimicrobial, protein-adhesive polymer patches, and nonfouling, protein repellent-polymer patches. By carefully balancing the size and architecture of the adhesive and repellent patches, materials with simultaneous antimicrobial activity and strong protein repellency are obtained. At similar polymer patch sizes, protein adhesion is lower on hydrogels with a low elastic modulus than on polymer monolayers attached to stiff substrates. Surface-regenerating polymer multilayer stacks are constructed from alternating layers of antimicrobial polymer hydrogels and degradable, soluble, or depolymerizable sacrificial layers. Top layer shedding, which imitates reptiles shedding their skin, rejuvenates the surface, and regenerates the antimicrobial function of the material. Layer shedding form such materials in solution is a competition between two thermodynamic minima, top layer reattachment and top layer removal. The outcome of each shedding event depends on the kinetics of the sacrificial layer disintegration.

1. Introduction

Seven years ago, our group was invited to contribute a research article to the special issue "Young Talents in Polymer Science" in *Macromolecular Chemistry and Physics*,[1] an honor that we gladly accepted. Today, we are giving a progress report of what happened since then in our work on antimicrobial and protein-repellent polymer materials.

At the time of that article with the title "Just Antimicrobial Is Not Enough", the group was just picking up speed with work on simultaneously antimicrobial and protein-repellent polymer materials. Before, we had worked on structure–property relationships of polycationic antimicrobial surfaces coated with cationic poly(oxanorbornenes),[2] which are synthetic mimics of antimicrobial peptides (SMAMPs, **Figure 1**a).[3–5] Like natural antimicrobial peptides (AMPs, Figure 1a),[6,7] SMAMPs are facially amphiphilic, i.e., they have hydrophilic, positively charged groups on one side, and hydrophobic groups on the other side of the molecule. They can selectively interact with the negatively charged cell envelopes of bacteria through their positive charges, and disturb the structure of bacterial membranes by interaction with their hydrophobic residues while leaving mammalian cells intact.[8,9] We had investigated the structure–property relationships of poly(oxanorbornene) SMAMPs as small molecules,[9,10] and studied their properties as surface-attached polymer networks.[2] There, we could correlate the pK_a value of these materials to their antimicrobial activity (Figure 1b),

M. Zober, K. Lienkamp
Department of Microsystems Engineering (IMTEK)
University of Freiburg
Georges-Köhler-Allee 105, 79110 Freiburg, Germany
E-mail: karen.lienkamp@uni-saarland.de

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M. Zober, K. Lienkamp
Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT)
University of Freiburg
Georges-Köhler-Allee 105, 79110 Freiburg, Germany

K. Lienkamp
Professur für Polymerwerkstoffe
Fachrichtung Materialwissenschaft und Werkstoffkunde
Universität des Saarlandes
Campus, 66123 Saarbrücken, Germany

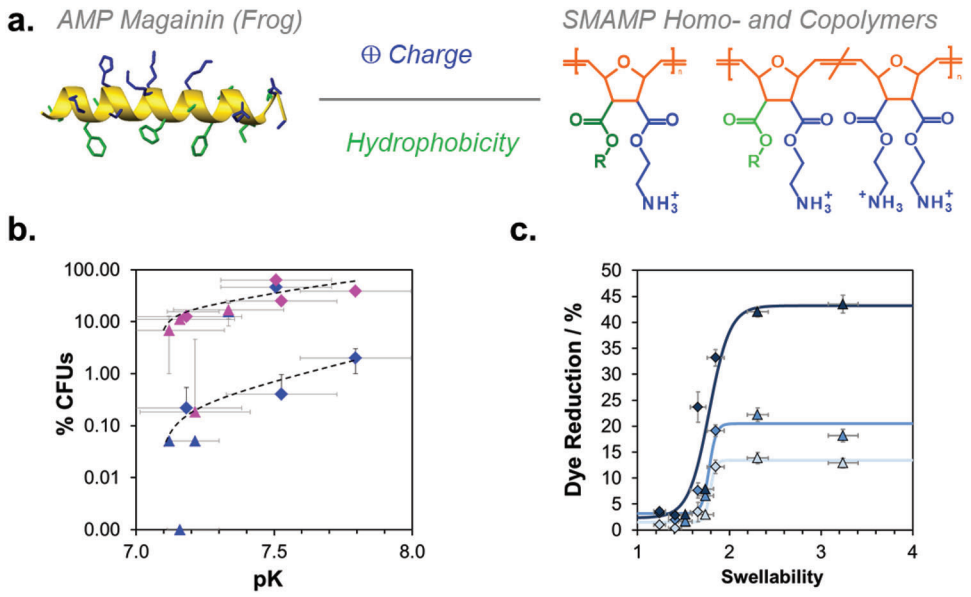

Figure 1. a) Like natural antimicrobial peptides (AMPs, e.g., magainin from frog skin), synthetic mimics of antimicrobial peptides (SMAMPs) are molecules that can organize into facially amphiphilic structures, with positive charges on one side, and hydrophobic groups on the other side of the molecule. b) The antimicrobial activity of surface-attached SMAMP networks (expressed as number of surviving colony forming units, CFUs) can be correlated with their acid constant (pK_a value) and thus the ratio of amine/ammonium groups on the surface; pink and blue symbols: activity against *S. aureus* and *E. coli*, respectively; diamonds: homopolymers, triangles: copolymers. c) The cell compatibility of the SMAMP networks with human kerationcytes (expressed as dye reduction, which is proportional to the cell's metabolic activity) is proportional to the materials' hydrophilicity (expressed as swellability in water). Light, medium, and dark blue symbols refer to data taken after 24, 48, and 72 h, respectively; diamonds: homopolymers, triangles: copolymers. Adapted with permission.[2] Copyright 2015, the Royal Society of Chemistry.

and their swellability (a measure for hydrophobicity) to their compatibility with mammalian cells (Figure 1c).[2] We also found that when surface-attached, the cell compatibility of SMAMPs was much better than as molecular entities,[2] a finding that has since then was confirmed by our collaborators when investigating SMAMPs covalently attached to nanoparticles.[11]

Thus, from an academic perspective, the design rules for SMAMP-like antimicrobial polymers and materials had been established. That, however, was only a first step toward potential applications as antimicrobial medical devices or antibiotics alternatives. Further detailed studies showed that for such applications, poly(oxanorbornene) SMAMPs still lacked sufficient chemical stability under application conditions and during ethylene oxide sterilization. But even if antimicrobial polycationic SMAMP materials would be sufficiently stable, in an open system bacteria would still eventually overgrow them. The reason for this is intrinsic to the properties and mode of activity of these materials: polycationic antimicrobial surfaces are contact active and attract oppositely charged biomolecules. Thus, they are easily contaminated by the negatively charged debris of dead bacteria, and incoming bacteria can settle on these contaminations without touching the still active SMAMP underneath.

By further improvement of their activity and/or killing capacity (e.g., by producing porous structures with high surface area as shown for a different structure by Chan-Park and co-workers),[12] the performance and period of activity of SMAMPs could possibly be extended, yet their final fate, i.e., being overgrown by bacteria on the long run, would remain unchanged. In other words, antimicrobial activity of polymer surfaces alone is not enough for long term activity, and alternative approaches are needed.

In the polymer field, it is well-known that "antifouling" polymer materials, i.e., polymers that prevent the formation of a biofilm, are attractive alternatives to intrinsically antimicrobial surfaces.[13,14] These passive materials form two groups: so-called "non-fouling materials" prevent the adhesion of proteins, bacteria or cells, while "fouling-release materials" such as poly(siloxanes) enable the removal of adhering biomolecules/organisms by shear forces, as has been reviewed extensively.[13–15] While these are promising properties to slow down the initial surface colonization and the maturation of biofilm communities, the downside of such approaches is that passive polymer surfaces are defenseless once the first bacteria have attached, e.g., by interacting with surface-adhering lipids or other (in)organic debris.

To combine the passive protection of "antifouling" polymers with antimicrobial activity in one material, polymer materials with both "antifouling" and antimicrobial moieties were synthesized, yielding surfaces with dual antibiofilm activity.[16–19] The typical strategy to obtain biofilm-reducing polymer coatings with dual activity was to load protein-resistant hydrophilic polymers with leaching antimicrobials, or to combine a protein-resistant polymer with a contact-active antimicrobial polymer.[16,20] In the latter case, it soon became clear that the surface architecture had a critical impact on the bioactivity obtained, as it determined if and to which extent each of the two components was bioavailable. As cationic antimicrobial materials are intrinsically protein-adhesive, it was particularly challenging to truly unite contact-active antimicrobial properties with protein-repellency. In our

research on dual-activity antimicrobial and protein-repellent materials with precise surface architecture, we therefore decided to focus on controlling the spatial distribution of the two components. This was achieved by combining surface patterning techniques with selective surface chemistry. By this approach, we could control both the relative fraction and the position of each polymer on the surface, as discussed in detail in the second part of this report.

In addition to the concepts discussed so far, other interesting approaches exist to prevent bacterial adhesion, proliferation, and biofilm formation, such as photodynamic approaches, antiquorum sensing, and fostering specific protein interactions.[15,21,22] Each of these approaches has yielded interesting materials with promising biological activity, at least in model studies. Yet, to our knowledge, so far there is no material that could claim permanent inertness to biofilm formation. This is due to the multitude of proteins and organisms which are present in natural aqueous environments, all of which can be part of different biofilm types. Seen in that light, it will be an extremely difficult, if not impossible task to develop a one-size-fits-all antibiofilm material, particularly if leaching or persisting toxic components are to be avoided.

However, if a surface cannot be protected from biofilm formation on the long run, why not equip the material with renewable surface properties? Following this approach, degradable antimicrobial, or protein-repellent polymer surfaces have been developed, where hydrolytic or enzymatic degradation of the polymer would renew the material surface.[15,23–25] These are often called "self-polishing" coatings and have been long known, for example in the context of the prevention of marine biofouling, as summarized previously.[26,27] Historically, "self-polishing" coatings contained heavy metals, in particular organo-substituted tin (e.g., tributyl tin, which is now banned for environmental reasons) or copper particles embedded into degradable polymer matrices.

The disadvantage of such approaches is that surface renewal is entirely kinetically controlled (e.g., by the degradation rates of the polymer used), and in consequence surface regeneration may not be uniform. Degradable polymers often contain fast-degrading amorphous regions as well as more stable crystalline domains. In practice, this leads to crater formation in the domains of the amorphous polymers, enhances the surface roughness, and enables bacterial adhesion in the crevices formed. To overcome this problem, our group developed a type of antimicrobial material able to rejuvenate its surface in a more organized way. This material was designed to shedding its top layer when contaminated, like a lizard shedding its skin. It consists of stacks of functional polymer layers and sacrificial interlayers. This concept is different to the well-known polyelectrolyte multilayer materials (PEMs) because it contains thicker, discrete polymer layers that are organized in a stack, like piled-up pancakes. In contrast, PEM layers are very thin and intertwined by the strong electrostatic interaction of the oppositely charged polyelectrolytes, and thereby resemble rather scrambled eggs that an ordered pancake stack.[28,29] As already discussed by Lynn and co-workers, peeling back layers individually from such PEM stacks was not possible due to these features.[30] With the more uniform interfaces—or rather interphases—of thick, discrete polymer layers of our polymer multilayer stacks, selective and sequential top layer shedding was possible, albeit not trivial. Our approaches to obtain such layer-shedding, self-regenerating materials are presented in the third part of this report, followed by a critical outlook onto the field.

2. Dual-Activity Antimicrobial and Protein-Repellent Polymer Surfaces

When designing dual-activity antimicrobial and protein-repellent polymer surfaces, the crucial question is how to control the ratio and distribution of the two components at the polymer-air or polymer-solution interface, so that both types of bioactivities are "seen" by their environment, and a dominance of the surface properties by one of the two components is avoided. "Grafting-from" or "grafting-onto" reactions are popular methods to obtain bifunctional polymer surfaces, however they provide only limited control over the surface architecture. In these methods, the ratio of the components can be controlled through the density of reactive sites on the surface (needed either for surface-initiated polymerization or as anchor groups for the surface grafts), and through the reaction kinetics of each reaction type. However, a nonuniform spatial distribution cannot be obtained by such methods alone. Yet fabricating dual-activity surfaces with distinct surface patterns, like the black and white fields of a chess board, and with different pattern dimensions, seemed like an efficient way precisely tailor the bioactivity of such materials. To that end, we combined surface structuring techniques with orthogonal, site selective surface chemistry to be able to direct each surface component to predetermined surface sites. The surface structuring techniques gave access to a chemical contrast on the surfaces, onto which specific anchor groups were immobilized. These were then used to immobilize two different polymer types selectively on designated sites of the surface patterns. The antimicrobial component used was from the previously described SMAMP polymer family (Figure 1), and the protein-repellent component was a nonfouling polyzwitterion with sulfobetaine groups (PSB, **Figure 2**). Each polymer type had a number-average molecular mass (M_n) of about 100 000 g mol^{-1}.

In the first approach, a chemical contrast on the substrate was created by colloidal lithography.[31–33] In this process, gold was evaporated onto a silicon background through a special lithographic mask—a self-assembled colloid monolayer (Figure 2a). This gave gold islands on a background of spherical silicon patches. In the following reaction steps, chemoselective anchor groups (lipoic acid-functionalized benzophenone, LSBP, which is selective for gold; and triethoxysilane-functionalized benzophenone, 3EBP, which is selective for the OH groups of the plasma-treated silicon substrates) were used to first immobilize one polymer (e.g., the SMAMP or its precursor) on the gold, and then the second polymer (e.g., the polyzwitterion PSB) on the silicon moieties of the substrate. The thus obtained materials consisted of a monolayer of the antimicrobial SMAMP on the gold islands, and a monolayer of the protein-repellent PSB the silicon background. This surface structure will be referred to as SMAMP@Au_PSB@Si in the following. Surface characterization of these materials by atomic force microscopy (AFM) indicated that the typical height of the gold islands was 30–50 nm, and that the monolayers had a thickness of 10–20 nm, which matches the diameter expected for surface-attached polymers with a M_n of 100 000 g mol^{-1} (Figure 2c). Further specifics of

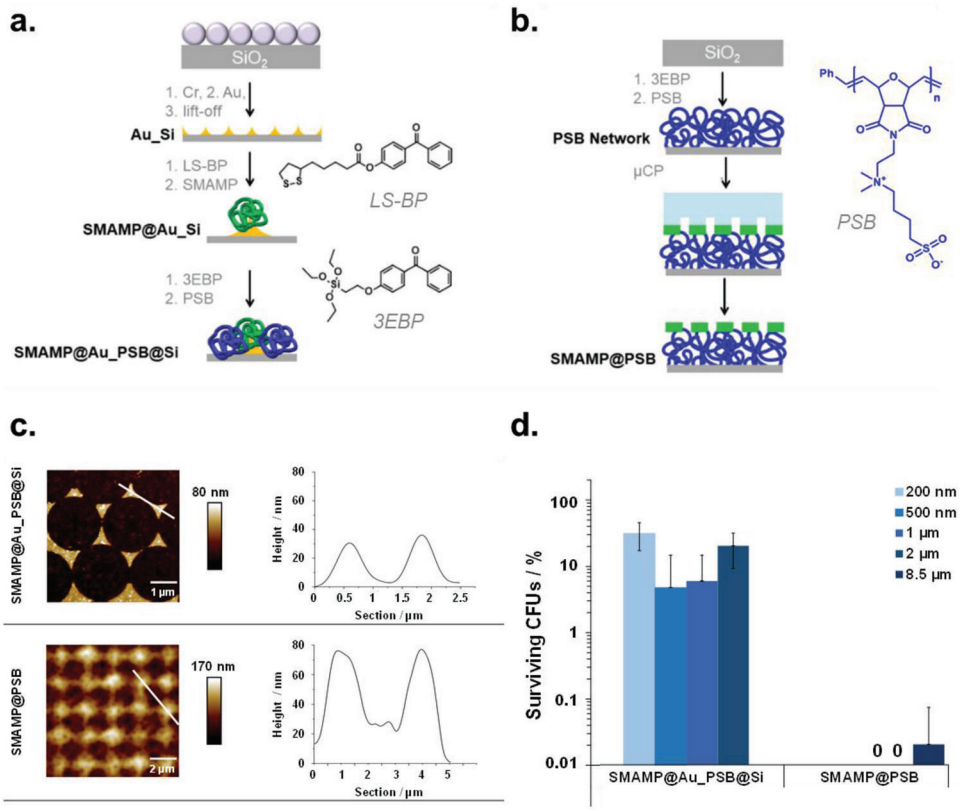

Figure 2. a) Fabrication process for the dual-activity polymer surfaces SMAMP@Au_PSB@Si by colloidal lithography. A colloid monolayer assembled on a silicon substrate was used as a lithographic mask through which first chromium (as adhesive layer), and then gold was evaporated. After removal of the colloids, gold islands on a silicon background were obtained. The gold islands were functionalized with gold-selective anchor groups (LS-BP), onto which SMAMP polymer monolayers were covalently immobilized by UV cross-linking. Functionalization of the silicon background with a silicon-selective anchor group (3EBP) yielded attachment points for covalent immobilization of a PSB polymer. b) Fabrication process for the dual-activity polymer surfaces SMAMP@PSB by microcontact printing. A surface-attached polymer network made from PSB was formed on 3EBP-functionalized silicon. A patterned poly(dimethylsiloxane) stamp inked with SMAMP was pressed onto the network to transfer SMAMP ink patches, which were simultaneously cross-linked and surface-attached by UV irradiation. c) Atomic force microscopy (AFM) height images and cross-sectional profiles of the material obtained by colloidal lithography (top) and the material obtained by microcontact printing (bottom). d) Antimicrobial activity (expressed as surviving colony forming units, CFUs, of *Escherichia coli* bacteria) of both types of material at different pattern spacings. (a Adapted with permission.[34] Copyright 2017, American Chemical Society, (b–d) Adapted with permission.[40] Copyright 2018, American Chemical Society.

the surface fabrication process and detailed surface characterization data has.[34–36] By varying the size of the colloids forming the mask (diameters used: 200, 500, 1000, and 2000 nm), bifunctional surfaces with different patch sizes were obtained.

In the second approach, the same bioactive polymers, SMAMP and PSB, were used. This time, the material consisted of a surface-attached polymer network made from PSB, onto which SMAMP patches were stamped by microcontact printing (Figure 2c).[37,38] To obtain this material, first, a thin layer of PSB (containing a few mol% of repeat units with built-in cross-linker groups) was spin-coated onto a 3EBP-functionalized silicon surface and simultaneously cross-linked and surface-attached by UV irradiation. A microstructured poly(dimethylsiloxane) stamp was then inked with a SMAMP solution (also containing a few mol% of built-in cross-linker repeat units) and pressed onto the PSB network to transfer the polymer. Further UV irradiation caused cross-linking of the SMAMP patches and, simultaneously, their covalent attachment to the PSB bottom layer. By using stamps with different spacings (1, 2, and 8.5 μm peak to peak distance), SMAMP patches with varied sizes could be obtained. An AFM image of a representative bifunctional material thus obtained (and referred to as SMAMP@PSB in the following) is shown in Figure 2c, together with its cross-sectional height profile. While the bifunctional material SMAMP@Au_PSB@Si obtained by colloidal lithography had a smooth, defined height profile, the surface structure of the bifunctional material SMAMP@PSB was much more rugged. At the same spacing of 2 μm, the SMAMP patches in SMAMP@PSB were about 80 nm high, and their phase boundaries were scraggy due to their network structure, which has a more uneven distribution of polymer segments at the interface than a polymer monolayer. Further details and a full materials characterization can be found in previous publications.[36,39,40]

The consequence of these differences in surface architecture are revealed by studying in the antimicrobial activity and protein repellency profiles of these materials. The antimicrobial activity against *Escherichia coli* bacteria of SMAMP@PSB was much higher than that of SMAMP@Au_PSB@Si (Figure 2d). At a

spacing of 1 and 2 μm, SMAMP@PSB quantitatively eliminated the bacterial load with which this surface was challenged. At a spacing of 8.5 μm, still more than a 3 log reduction (> 99.9% killing) could still be observed. In contrast, the SMAMP@Au_PSB@Si surfaces showed only about one log reduction (90% killing), with a somewhat better antimicrobial activity at spacings of 500 nm and 1 μm nm, while the materials with the smallest (200 nm) and largest (2 μm) spacings performed poorest. Thus, these two types of materials, which consisted of the same polymers and featured similar pattern spacings, had a distinctly different bioactivity profile. AFM images indicate that the surfaces obtained by colloidal lithography consisted of polymer monolayers with a comparatively smooth, homogeneous polymer segment density at the interface, and thus had a comparatively well-defined phase boundary. Approaching bacteria would preferentially settle on the SMAMP patches of these materials, where they would experience electrostatic attraction. When the spacing had the same dimensions as the bacteria, the contact area of bacteria and these patches was highest, and so was the antimicrobial activity. When the patches were smaller, the antimicrobial activity was lower, potentially because the SMAMP patches were too small to sufficiently damage the membrane. At the largest spacing of 2 μm, the activity of the SMAMP@Au_PSB@Si materials was also lower. Here, some bacteria could settle directly at the SMAMP-PSB site boundaries, where they could safely adhere without significant membrane damage. The polymer networks SMAMP@PSB, on the other hand, had a much more inhomogeneous segment density, as evidenced by the AFM images. In the areas with lower segment density, the polymers have a higher chain mobility, especially with increasing distance to the underlying substrate. Also, the polymer networks were thicker and softer (as evidenced by quantitative nanomechanical AFM measurements, data not shown).[36] Thus, it is plausible that bacteria interacting with the SMAMP@PSB network patches gain less adhesion energy near the SMAMP patches, even if these patches are sufficiently large, because they are unable to establish a sufficient number of contacts simultaneously. Also, the incoming bacteria reduce the volume accessible to the dangling polymer chain ends, which reduces the conformational entropy of the polymer patches, and thereby further contributes to an unfavorable overall free enthalpy of adhesion. At the same time, although not adhering, the incoming bacteria are in temporary contact with the dangling antimicrobial polymer chains of the networks, which seems to cause sufficient bacterial membrane damage to reduce their growth rate. Another reason for the overall better antimicrobial performance of SMAMP@PSB compared to the corresponding SMAMP@Au_PSB@Si monolayers could be that these thicker layers are less prone to have surface defects which would allow bacteria to settle and proliferate.

The protein repellency on the two types of materials is also different (data not shown).[36] On the SMAMP@Au_PSB@Si monolayers, no protein adsorption was observed for materials with a spacing of 200 and 500 nm, while the materials with the larger spacings of 1 and 2 μm had an average protein adsorption of up to 0.5 ng mm^{-2}.[36] The SMAMP@PSB networks, on the other hand, showed no protein adhesion at all spacings of 1, 2, and 8.5 μm.[36] Thus, the interface of the latter material was not even adhesive enough for proteins, which are roughly one order of magnitude smaller than bacteria, in spite of the opposite charges of the protein used and the SMAMP patches. This can be explained with the same line of argument as presented for the antimicrobial activity, namely with insufficient adhesion energy in comparison to the configurational enthalpy lost if protein would adhere.

So why does the approach to include adhesive, antimicrobial and protein-repellent polymer patches in one material for simultaneous dual activity work at all? Is not this kind of material a contradiction in itself? How should a surface containing cationic, adhesive polymers be nonfouling and protein-repellent at the same time? After all, antimicrobial activity requires sufficient contact between the bacteria and the surface, while nonadhesive surfaces are designed so that nothing, especially not proteins, should be able to adhere. When we look at these materials statically, it is certainly an oxymoronic concept. Yet polymer surfaces are not static objects. They are highly dynamic materials, their chains and/or chain segments are in perpetual motion, particularly, if the free volume inside the polymer is as large as it is in polymer hydrogels. Thus, the polymer hydrogel patches do not have a sharp phase boundary to the liquid phase, and any interactions between the hydrogel surface and objects from the outside must take the dynamics of both the adhesive, antimicrobial and the protein-repellant moieties into account. One can consider the situation on the surface of such bifunctional materials as an array consisting of oppositely directed force fields—one attractive, one repulsive. When the proportions and dimensions of the two moieties (relative to the incoming object) are chosen just right, these force fields compensate each other, yet locally and temporarily, the attractive component can dominate, so that bacteria can get into contact with the surface. These short spatial and temporal fluctuations seem to be sufficient for the cationic antimicrobial patches to destabilize the bacterial membrane and cause leakage of the bacterial cytoplasm. However, they are insufficient for establishing a thermodynamic equilibrium in which the bacteria or proteins gain sufficient free enthalpy of adhesion, so that the interaction stays reversible. This interpretation would also explain why SMAMP@Au_PSB@Si showed a different spacing-activity threshold than SMAMP@PSB: On the SMAMP@Au_PSB@Si materials, which are more densely packed than the hydrogels, the entropic effect is less pronounced than on SMAMP@PSB and cannot overcompensate the adhesion energy of bacteria on the SMAMP patches.

3. Self-Regenerating Polymer Surfaces

As discussed in the Introduction, it is difficult to keep antimicrobial and protein-repellent polymer surfaces free from contamination by biofilms, and to extend their lifetime ad infinitum. As an alternative, we investigated antimicrobial materials featuring various surface self-regeneration mechanisms.[25,41,42] In particular, we developed antimicrobial materials that were polymer multilayer stacks. These were made from discrete, tens to hundreds of nanometer thick individual polymer layers (not to be confused with PEMs, as discussed in the Introduction). For surface regeneration after contamination, such stacks should be able to shed their top layer, like a lizard shedding its skin (**Figure 3**a). The desired layer properties for the stack were antimicrobial activity, low roughness, good cohesion, and sufficient adhesion to the layer underneath. Additionally, it should be possible to selectively

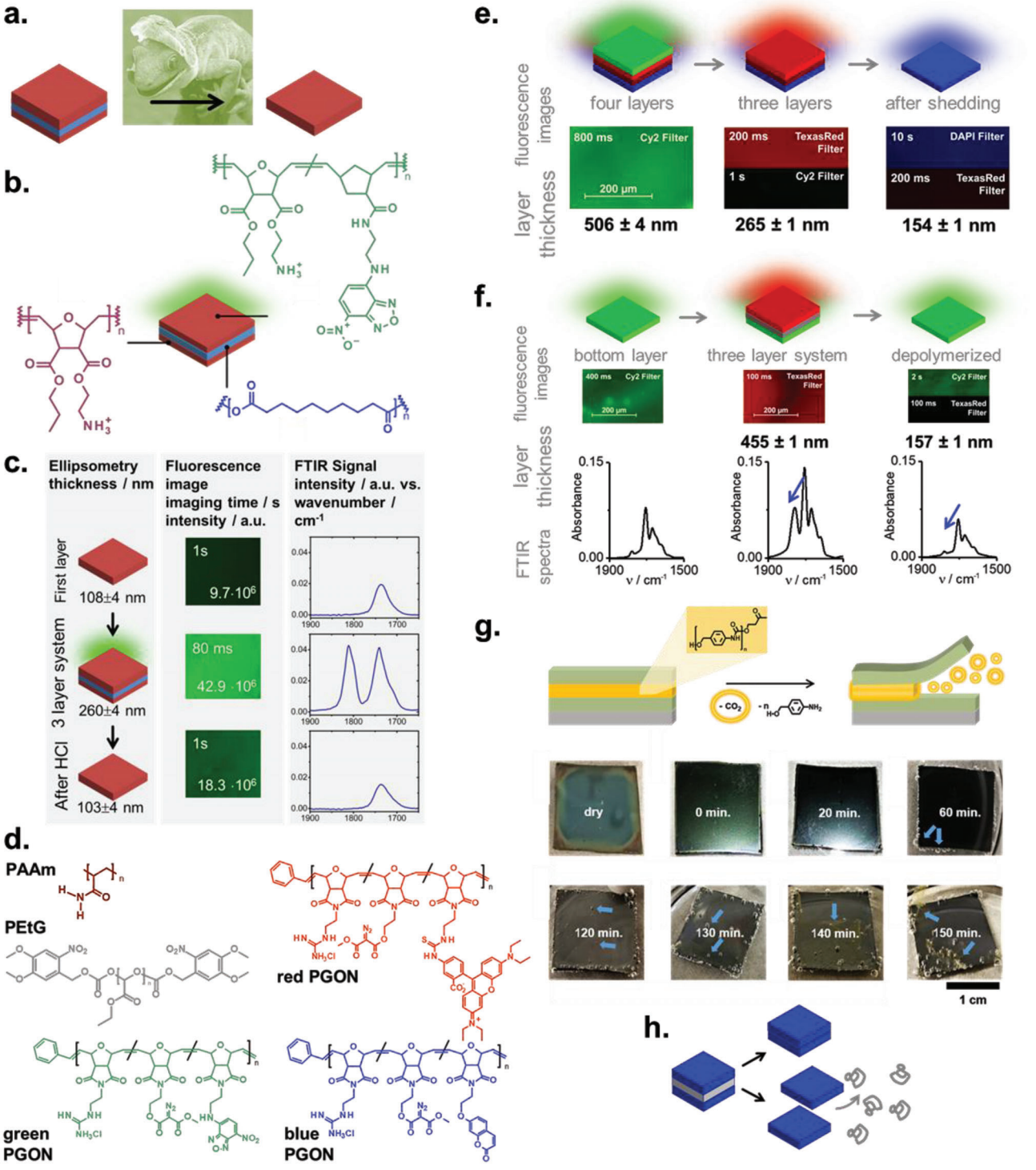

Figure 3. a) Self-regenerating polymer surfaces consisting of functional layers (red) and decomposable interlayers (blue). By decomposition of the interlayers, they can shed their top layer, like a lizard shedding its skin. A model stack made from antimicrobial polymers and degradable PSA b) could shed the top layer only in warm HCl c), but not under physiological conditions. Other polymer multilayer stacks consisted of antimicrobial PGON hydrogels with different fluorescent labels, and soluble PAAm or depolymerizable PEtG interlayers d). e) Multilayer stack with two soluble layers (green PGON and PAAm), featuring sequential layer shedding. f) Triggered layer shedding using PEtG with UV-cleavable end groups. g) Poly(benzyl carbamate) (PCB) as sacrificial layer. Carbon dioxide is released during PCB depolymerization. The additional buoyancy forces help the layer shedding process. h) Layer shedding has two energetic minima—one for readhesion, one for shedding. The disintegration kinetics of the sacrificial layer determine the fate of the system.(a–c) Adapted with permission.[42] Copyright 2015, American Chemical Society; (d–f) Adapted with permission.[48] Copyright 2019, Wiley-VCH GmbH. (g,h) Adapted with permission under the terms of the CC-BY license.[49] Copyright 2021, the Authors. Published by Wiley-VCH GmbH.

modulate the interlayer adhesion between the top layer and the second layer without affecting the adhesion of the following layers. As this latter requirement is difficult to fulfill if the layers are all made from the same material, it seemed much easier to construct the target system from alternating functional and sacrificial layers (Figure 3a).[42] This concept was borrowed both from nature and from microsystems fabrication: in the latter, a sacrificial layer is a soluble polymer layer which can be applied as a spacer during the fabrication of microstructures, and is later dissolved to retrieve the target microsystem.[43] For our polymer multilayer stack, the sacrificial layer was designed in such a way that it had sufficient non-covalent interactions with the adjacent functional layers to form a stable material, but could be fully disintegrated to enable shedding of the top layer. This design greatly simplifies the problem of layer shedding: instead of having to break the adhesive interactions between two connected layers, with a sacrificial layer the interface between that layer and the two adjacent functional layers gradually disappears due to the molecular-level disintegration of the sacrificial layer in the surrounding medium. During degradation, the liquid surrounding the polymer stack would flow into the forming void, and thereby prevent reattachment of the shedding functional layer to the remaining stack. In this process, two polymer-polymer interfaces are replaced by two polymer-solvent interfaces. While this is energetically unfavorable because it increases the overall surface energy of the system, it is entropically favorable due to the entropy gained both by the released top layer and the sacrificial layer components.

Different polymers were designed and evaluated as sacrificial layers for these polymer multilayer stacks. The first group of materials studied were degradable poly(anhydrides) and poly(ester anhydrides).[42,44] When immersed into aqueous media, these polymers hydrolyze into diacids and hydroxyacids, respectively. In a proof-of-concept system, an interlayer made from poly(sebacic anhydride) (PSA) was sandwiched between two antimicrobial SMAMP hydrogel layers (Figure 3b). Cohesion of the hydrogel layers was established by covalent cross-links within the layer (formed either by a thiol-ene-reaction with a low molecular mass cross-linker like pentaerythrit-tetrakis-(3-mercaptopropionat), or by inter- and intramolecular C,H-insertion reactions with built-in co-repeat units containing UV-activated cross-linker groups). The thermoplastic PSA layer was mechanically stable in consequence of its crystalline domains. Dipolar interactions between the ester groups of PSA and the polar groups of the SMAMP provided sufficient adhesion between the layers.

When immersed into hydrochloric acid at 60 °C, the PSA layer degraded fast (within less than 40 min), so that the adhesive interactions were broken, and shedding of the topmost SMAMP layer was observed. This was evidenced by fluorescence microscopy, Fourier-transform infrared spectroscopy (FTIR) and ellipsometry (Figure 3c). The thereby uncovered SMAMP layer was antimicrobial, i.e., the amount of PSA debris left behind was negligible and did not compromise the performance of the material.[42] However, when immersed in water at neutral pH, degradation took much longer (several days), and no layer shedding was observed. Instead, while the PSA layer degraded, the topmost functional layer reattached to the stack. In order to understand this phenomenon and to obtain a system that could also shed the top layer under physiological conditions, we studied the degradation kinetics of noncrystalline poly(ester anhydrides), poly(anhydrides), and blends of different poly(anhydrides), which disintegrated substantially faster than PSA.[44] Unfortunately, the results were always the same: when degradable polymers were used as sacrificial layers under physiological conditions, the top functional layer would reattach to the polymer stack. We arrived at the hypothesis that although the sacrificial layer had vanished in all these cases (which was clearly seen by FTIR spectroscopy), no shedding was observed because the adhesive interactions between the sacrificial layer and the functional layer were not broken in a sufficiently concerted process. As a result, a substantial influx of the surrounding medium between the layers was prevented, and the flexible top layer reattached to minimize the surface energy of the system. Thus, we had to give up the initial plan to assemble polymer multilayer stacks from SMAMP hydrogel layers and degradable polymers with different degradation rates, so that sequential layer shedding could be observed.

At the first glance, it is conceptually difficult to understand why the topmost polymer layers would not delaminate from these multilayer stacks. Typically, delamination of polymer coatings from materials surfaces (e.g., wood or metals) is an undesired process which needs to be prevented by careful design of the material-coating interface—yet our system which was explicitly designed for delamination could not shed its layers. Closer inspection of these two situations explains these different results: wood and metals are hard, rigid substrates. When polymer coatings on such substrates undergo significant changes in environmental conditions (e.g., temperature changes), a property mismatch (e.g., in their thermal expansion coefficients) may cause a build-up of mechanical stress at the polymer-substrate interface. As a result, cracks are formed, humidity enters the interface, and the coating delaminates. The situation is quite different in the case of sacrificial layers mounted between two soft, flexible polymer hydrogels. While the sacrificial layer degrades, degradation products slowly diffuse out of the interfacial area, the hydrogels can form new non-covalent bonds to the remaining sacrificial polymer layer, and thereby the system adapts to the new interfacial situation. The dynamics of this interface and its flexibility prevent the build-up of interfacial tension. The reattachment process is potentially also driven by thermodynamics, for example the increase in surface energy associated with layer shedding may not be sufficiently compensated by entropic effects when the sacrificial layer fragments are released too slowly.

As polymer degradation under physiological condition was too slow to successfully shed layers from polymer multilayer stacks, the following studies focused on sacrificial layers with different disintegration mechanisms: simple dissolution, and triggered depolymerization. Water-soluble poly(acrylamide) (PAAm, Figure 3d) was used as a proof-of-concept sacrificial layer to demonstrate that delamination of soft polymer hydrogels as such is feasible. PAAm and poly(vinyl alcohol) are common sacrificial layer materials in microsystems fabrication, where they are used to release structures made from hard matter like silicon, glass, or rigid polymers. In combination with PAAm, we used an antimicrobial guandinium-substituted polymer (PGON, Figure 3d) for the hydrogel layers, which had repeat units containing different kinds of fluorophores to facilitate visualization of the layer shedding, in addition to repeat units with UV cross-linker groups. Additionally, PGON was more stable against hydrolysis than the amine

group-containing SMAMP polymer. Initial studies showed that the topmost PGON layer could be shed from the three-layer system PGON-PAAm-PGON under physiological conditions (data not shown). The dissolution of PAAm under physiological conditions took 20–30 min, which was substantially faster than PSA degradation, and thus the detachment of the adhesive interactions to the PGON layer were also more synchronous. Thus, the flexibility of soft hydrogel layers as such, i.e., their ability to dissipate mechanical stresses at the layer interface, did not prevent the layer shedding process.

In the next step, we assembled a four-layer stack of green fluorescent, not cross-linked PGON as the top layer, red fluorescent, cross-linked PGON, PAAm, and blue fluorescent, cross-linked PGON (attached to a silicon substrate) as the bottom layer (Figure 3d,e). With this stack, we could demonstrate selective and sequential layer shedding by fluorescence microscopy (Figure 3e): the green-fluorescent PGON layer (the topmost antimicrobial layer) was shed immediately after contact with water, revealing the second, red-fluorescent functional PGON layer. After 15 min, the PAAm sacrificial layer was dissolved and released the next PGON layer, i.e., the red fluorescence fully vanished, so that the third, blue PGON layer was uncovered (Figure 3e). Such a system can thus clear contamination at the surface twice, and thereby potentially enhances the lifetime of an antimicrobial medical device, e.g., a catheter tube. Of course, the time intervals of the layer release in this example are yet too short, and, as they depended on the relative dissolution rates of PGON and PAAm under physiological conditions, they cannot not be controlled further. Yet this study was important to demonstrate that layer shedding of soft, compliant hydrogel layers under physiological conditions is feasible, and that sequential layer shedding could also be realized.

Based on these results, it was evident that the ideal sacrificial material should be able to disintegrate fast (i.e., within a few minutes), and contain a built in trigger to be able to initiate the process at an arbitrary time point. To achieve this, we borrowed ideas from the field of self-immolative polymers for the sacrificial layer concept. Self-immolative polymers are stabilized above their ceiling temperature by a suitable end-group. Once this end-group is cleaved, the polymer undergoes head-to-tail depolymerization.[45–47] Thus, self-immolative polymers that allow end-group cleavage by external stimuli are ideal candidates for triggered sacrificial layer disintegration. We first studied poly(ethyl glyoxylates) (PEtG) with UV-cleavable 6-nitroveratryl carbonate end-groups as sacrificial layers.[48] A polymer three-layer stack consisting of a red-fluorescent top layer, the PEtG sacrificial layer, and a green-fluorescent bottom layer was assembled (Figure 3f). Two hours after UV irradiation, the red layer was shed (i.e., the red fluorescence of the material vanished), and the green layer became the outermost functional layer, indicating successful layer shedding under simulated physiological conditions. We also used poly(benzyl carbamate) (PBC) as sacrificial layer with an additional driving force for shedding: when degrading, this polymers releases carbon dioxide. The gas forms bubbles at the material interface and thus has a buoyancy effect on the attached top layer (Figure 3g).[49] When sufficient noncovalent bonds between the depolymerizing PBC layer and the top layer are broken, the buoyancy forces assist the top layer shedding, so that it can be removed, even though PBC depolymerizes significantly slower than PEtG (60–150 min, depending on the PBC layer thickness).[49]

In summary, we investigated three different mechanisms by which sacrificial layers used in polymer multilayer stacks can disassemble and thereby facilitate layer shedding of soft, compliant polymer layers: hydrolytic degradation, dissolution, and triggered depolymerization. In the systems studied, the outcome of the disassembly event depended on the timing and concertedness of the disassembly. In the case of slow disintegration, the top layer reattaches to the stack, but it can be successfully shed if the sacrificial layer disintegration is assisted by further external forces, e.g., buoyancy forces (Figure 3h). Thus, there are two free energy minima which the system can target, while the sacrificial layer disintegrates: either top layer reattachment or top layer shedding (Figure 3g), and the pathway the system takes seems to depend on the release rate of the sacrificial material.

The question often asked when we present this approach is about the sustainability of the concept. The intention of this research is to use these materials, on the long run, to increase the lifetime of a device and thereby reduce the frequency of repairs and replacement. However, do not we produce polymer fragments and microplastics by layer shedding, which could potentially bioaccumulate? For the currently used systems, the answer is yes. While some sacrificial layers were biodegradable or depolymerized to nontoxic organic molecules, the shed layers were polymer hydrogels which have so far not been optimized for degradability. This would be the next development step. When all polymer layers eventually become degradable (where the degradation kinetics of the layer to be shed is of less importance, provided that it is not persistent in the environment), the approach is sustainable, especially when comparing the environmental footprint of surface regeneration with that of replacing an entire device.

4. Conclusion and Outlook

In the past 5 years, our group has made substantial progress in the field of antimicrobial polymer surfaces with hierarchical polymer architectures. We have investigated the structure–property relationships of polymer surfaces made from antimicrobial and protein-repellent components, and demonstrated that the polymer patch sizes, the architecture of the polymer-liquid interface, and the mechanical properties of the polymer materials had an impact on the bioactivity of the materials. We could show that the properties of such materials could even be balanced between antimicrobial/adhesive and protein-repulsive, and thereby unite two properties that were previously considered as mutually exclusive. By assembling polymer multilayer stacks from functional and sacrificial layers, where the latter could disintegrate via different mechanisms, we demonstrated that it is possible to regenerate surface properties of polymers selectively, sequentially, and by external triggers. From a scientific perspective, these materials are unprecedented and therefore interesting. Whether they will find their way into any applications will depend on other factors: stability under conditions of storage and use, and ease and cost of fabrication. So far, some of the processing techniques used only work for flat substrates and/or are difficult to scale up. Thus, it will depend on the target application, or the possibility to simply the fabrication of these or similar materials, to decide if it is

worthwhile to pursue these approaches further to obtain real life materials.

Ironically, after working for years on multicomponent materials with complicated architectures, we recently discovered a one component material, specifically a family of polyzwitterionic hydrogels,[50–52] which had antimicrobial activity in bacterial assays, and was at the same time protein-repellent under physiological conditions. These materials were also highly cell-compatible, in some cases combined with slight cell adhesiveness, in others with cell repellency. At first glance, these polyzwitterions seemed to have well-balanced antimicrobial and protein-repellent properties. Further analysis by surface zeta potential measurements revealed that they were in fact switchable. In the surroundings of bacteria, which secrete acidic metabolites, they are protonated, adhesive, and antimicrobial. In the presence of a pH-neutral protein solution, they become protein-repellent.[50–52] Materials that were deliberately designed to switch their properties from antimicrobial to protein-repellent and vice versa were first presented by Jiang and coworkers.[53,54] However, in this system the antimicrobial activity needed to be regenerated by relatively harsh conditions (hydrochloric acid), and ex situ. In the system discovered by us, the same was achieved with simple protonation starting at pH 5. Since the first serendipitous example reported,[50] we deliberately searched and designed others,[51,52] and could show that polyzwitterions whose structures were similar to SMAMP-like polycations showed the desired pH-dependent switchability from antimicrobial to protein-repellent. Stimulus-responsive antimicrobial polymer materials have also been reported by other groups, and are expected to remain an intensively researched field in the near future.[55,56]

Where will the field go to from here? Many of the fundamental design rules both for antimicrobial activity and protein repellency seem to be understood. What still hampers their large-scale application in the biomedical industry are, besides cost, mainly practical problems. Most research systems have not been optimized for stability. They work well in situ and during the duration of laboratory assays but fail under conditions that medical devices have to sustain to be of practical use—they have insufficient stability under application or sterilization conditions, or only a short shelf-life. Once these problems have been overcome, we should see these materials in applications, but this is a matter of application-driven development and not of academic basic science.

With this report or our progress in the past 5 years, including some tales of failure and serendipity, we hope we could give the next generation of young talents in polymer science some insight into the joys and struggles of a new PI and her group. So what's next for us? Fundamental experiments with bioactive polymer surfaces will certainly keep us busy for some more time. In addition, we have turned to exploiting the chemistries developed for the above purposes to the fabrication of smart hydrogels for 3D printing. This is a field that is certainly going to keep us happy and excited for the next 5 years. Please get back to us in 2027, in the special issue on "Middle-aged Talents in Polymer Chemistry."

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Conflict of Interest

The authors declare no conflict of interest.

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Karen Lienkamp studied physical natural sciences and chemistry at the University of Cambridge and the Freie Universität Berlin. She then worked at the Max Planck-Institute for Polymer Research towards her Ph.D., and graduated at the Johannes Gutenberg-Universität in Mainz. She was a postdoc at the University of Massachusetts in Amherst, MA. Afterwards, she headed a research group at the Department of Microsystems Engineering of the Albert-Ludwigs-Universität Freiburg, where she received the Venia Legendi in 2017. Since 2021, she is a full professor for polymer materials at Universität des Saarlandes in Saarbrücken.