

Review

Antimicrobial Polymers in Solution and on Surfaces: Overview and Functional Principles

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Abstract: The control of microbial infections is a very important issue in modern society. In general there are two ways to stop microbes from infecting humans or deteriorating materials—disinfection and antimicrobial surfaces. The first is usually realized by disinfectants, which are a considerable environmental pollution problem and also support the development of resistant microbial strains. Antimicrobial surfaces are usually designed by impregnation of materials with biocides that are released into the surroundings whereupon microbes are killed. Antimicrobial polymers are the up and coming new class of disinfectants, which can be used even as an alternative to antibiotics in some cases. Interestingly, antimicrobial polymers can be tethered to surfaces without losing their biological activity, which enables the design of surfaces that kill microbes without releasing biocides. The present review considers the working mechanisms of antimicrobial polymers and of contact-active antimicrobial surfaces based on examples of recent research as well as on multifunctional antimicrobial materials.

Keywords: antimicrobial polymers; antimicrobial surfaces; biocides; bacteria; fungi

1. Introduction

The control of the growth of microbes, such as bacteria, fungi, yeast and algae, in nature is one of the fundamental concepts for the survival of higher species. Plants, animals, even microbes themselves have developed a great variety of mechanisms that keep microbes at bay [1]. In human society these

control mechanisms often do not work efficiently, which makes microbial infections the number one killer in the world. The treatment of microbial infections becomes more and more difficult, because the number of resistant microbial strains as well as that of antibiotic-immune patients grows a lot faster than the number of useable antibiotics [2,3].

Antimicrobial polymers represent a class of biocides that has become increasingly important as an alternative to existing biocides and in some cases even to antibiotics. The working mechanism of the large number of structurally different polymers is often not fully understood. However, some of them are known for their low potential of building up resistant microbial strains [4].

Man-made materials completely lack defence against microbial growth. Thus, microbial cells attached to any artificial surface in a moist environment can survive and proliferate. While the cell number increases on the surface the microbial cells usually start to build up a biofilm, which consists of a polysaccharide matrix with embedded cells (see Figure 1). Such biofilms allow microbial cells to survive under harsh conditions and the embedded cells are up to 1,000 times less susceptible to most antibiotics and other biocides [5]. Further, many toxins excreted from biofilms make the latter pathogenic and resilient infections are spread [6]. Further, antibiotic genes can be exchanged between bacteria within biofilms, which additionally enhances the formation of multi-resistant bacterial strains [7]. A recent example is the enterohemorrhagic *Escherichia coli* (EHEC) epidemic in Europe [8]. Additionally, almost every man-made material can be degraded by microbial biofilms [9]. Therefore, the control of microbial growth on surfaces is one of the key issues in material science as well as medicine.

Figure 1. Typical biofilms: (a) mold in households, (b) algae growth on a ship's hull, (c) bacterial biofilm on a catheter.



One way to prevent surface contamination is to keep the environment sterile, for instance by using disinfectants, such as hypochlorite, hydrogen peroxide or other reactive oxygen species (ROS). Alternatively silver salts, quaternary ammonium compounds or alcohols are in use. Another widely used disinfectant is triclosan. Unfortunately, the sterile state does not last for long and the frequent use of such disinfectants poses a great environmental problem, particularly dramatic in the case of triclosan [10]. Further, disinfectants have been shown to support the formation of resistant antimicrobial strains [11,12], e.g., methicillin resistant *Staphylococcus aureus* (MRSA), which causes the majority of nosocomial infections and notably causes more deaths in the USA than HIV [13].

Antimicrobial surfaces that prevent the growth of biofilms are an alternative way to inhibit the spread of microbial infections. Such surfaces either repel microbes, so they cannot attach to the

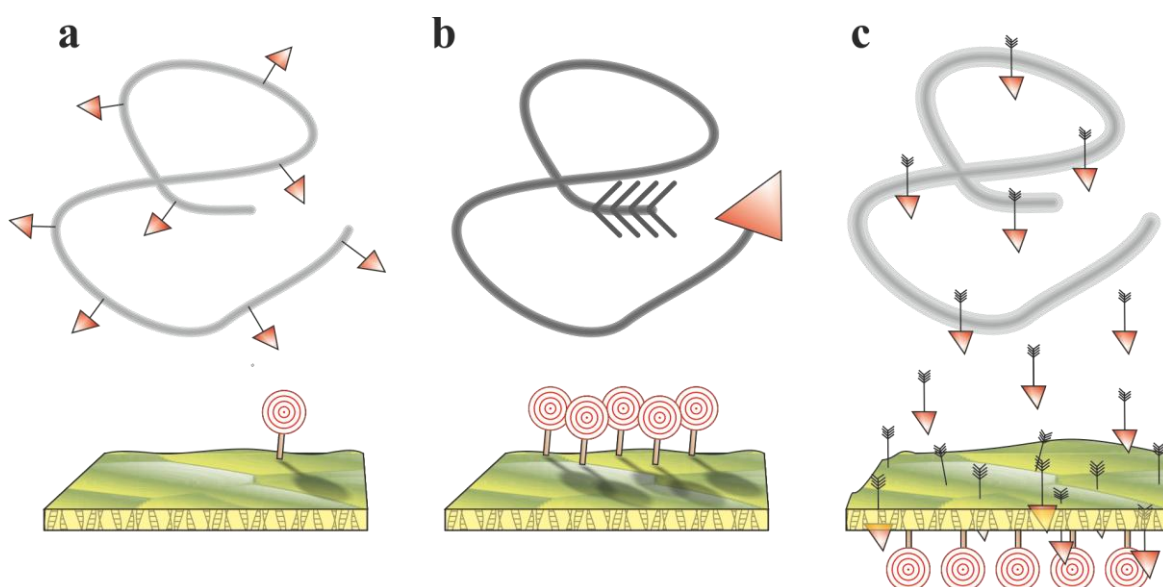
surface, or they kill microbes in the vicinity. The latter is achieved by surfaces loaded with biocides, such as silver, antimicrobial ammonium compounds, antibiotics, active chlorine, or triclosan [14]. Although such surfaces work quite efficiently, they eventually exhaust and the released biocides cause the above described problems. Alternatively, surfaces that catalytically produce biocides using externally applied chemical, electrical, or optical energy are an intriguing alternative (recently reviewed in [15]). Another possibility is the design of surfaces that kill microbes on contact, *i.e.*, they do not release biocides and do not exhaust, at least in principle. Such contact-active antimicrobial surfaces can be realized by tethering antimicrobial polymers [16]. Studies suggest that the polymers on these surfaces work differently to those in solution, whilst their true functional principle is still under speculation [17].

The present study gives an overview on recent developments in the field of antimicrobial polymers with particular focus on their function both in solution and on surfaces.

2. Antimicrobial Polymers

Antimicrobial polymers have been known since 1965, when Cornell and Dunraruma described polymers and copolymers prepared from 2-methacryloxytroponones that kill bacteria [18]. In the 1970s several groups synthesized various polymeric structures that showed antimicrobial action, *e.g.*, Vogl *et al.*, who polymerized salicylic acid [19], or Panarin *et al.* who synthesized polymers with quarternary ammonium groups [20]. A large number of such macromolecules are known to date. Their function is not always understood. Nevertheless, the number of FDA-approved disinfecting polymers has significantly increased in the past decade, which indicates the need for alternatives to antibiotics and environmentally critical disinfectants. Recently, several reviews have summarized the state of the art [15,21–24]. The most recent review of Timofeeva *et al.* thoroughly discusses the influence of relevant parameters, such as molecular weight, type and degree of alkylation, and distribution of charge on the bactericidal action of antimicrobial polymers [23].

Figure 2. General working principles of antimicrobial polymers: (a) polymeric biocides; (b) biocidal polymers; (c) biocide-releasing polymers.



Careful study of the literature-known antimicrobial macromolecules reveals three general types of antimicrobial polymers: polymeric biocides, biocidal polymers, and biocide-releasing polymers. As shown in Figure 2, the first class is based on the concept that biocidal groups attached to a polymer act in the same way as analogous low molecular weight compounds, *i.e.*, the repeating unit is a biocide. Taking into account the sterical hindrance exerted by the polymer backbone, polymeric biocides should be less active than the respective low molecular weight compounds. For biocidal polymers on the other hand the active principle is embodied by the whole macromolecule, not necessarily requiring antimicrobial repeating units. Biocide releasing polymers do not act through the actual polymeric part, instead the latter functions as carrier for biocides that are somehow transferred to the attacked microbial cells. Such polymers are usually the most active systems, because they can release their biocides close to the cell in high local concentrations.

2.1. Polymeric Biocides

Polymeric biocides are polymers that consist of bioactive repeating units, *i.e.*, the polymers are just multiple interconnected biocides, which act similarly to the monomers. Often, the polymerization of biocidal monomers does not lead to active antimicrobial polymers, either, because the polymers are water-insoluble or the biocidal functions do not reach their target. For example, the polymerization of the antimicrobial 4-vinyl-N-benzylpyridinium chloride and subsequent crosslinking resulted in a non-biocidal water-insoluble polymer that only captures microbes but does not kill them [25].

The polymerization of antibiotics, which is usually performed to obtain prolonged activity and less toxicity, is very sensitive to the tethering method and the nature of the antibiotic. For instance, Nathan *et al.* showed that the antibiotics Penicillin V and Cephadrine are not active when conjugated with PEG-Lysine via a hydrolytically stable bond, indicating that the polymer itself is not active [26]. However, the conjugates exhibited full antimicrobial activity if the biocides were tethered via a hydrolytically labile bond, which proved that the antibiotics are reactivated upon release. On the other hand recent examples indicate the possibility to polymerize antibiotics retaining their activity at the polymer backbone, *e.g.*, by copolymerization of methacrylate modified Norfloxacin and PEG-methacrylates [27]. Direct modification of Vancomycin with PEG-methacrylate and subsequent polymerization resulted in active polymers that are at least 6-fold less active when comparing them to the unmodified antibiotic [28]. The activity decreased with greater PEG-length.

These examples show the negative effect of PEG on most biocides, which was also found for quarternary ammonium groups attached to the polyether [29]. Interestingly, it was reported that penicillin attached to polyacrylate nanoparticles showed higher activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) than the free antibiotic in solution [30]. Polymers with antimicrobial side groups, based on hydrophobic quarternary ammonium functions can be considered as polymeric biocides as well [31]. However, some of those polymers are more active than the respective monomers, indicating that the mode of action is not that of the biocidal group alone.

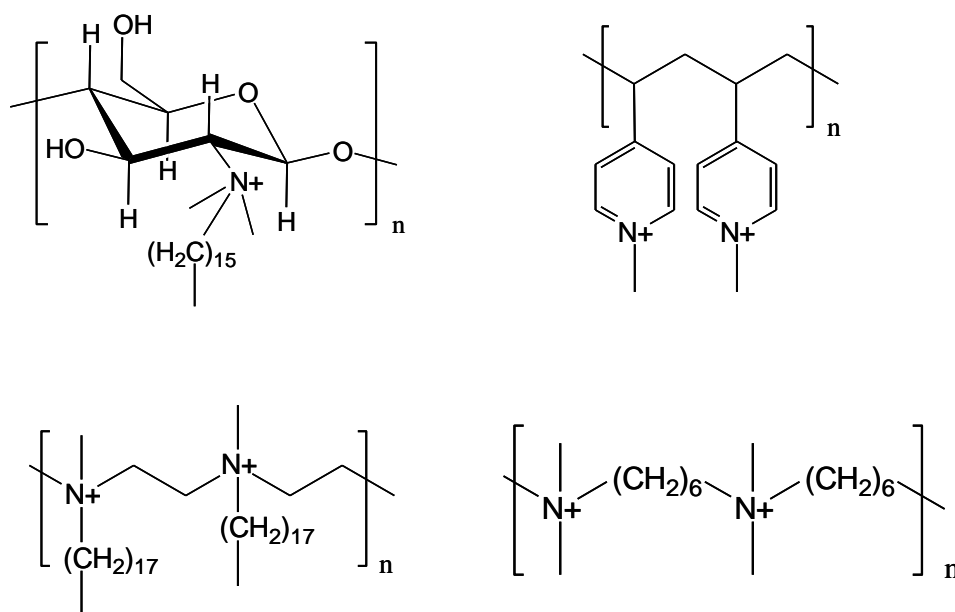
2.2. Biocidal Polymers

As pointed out by Timofeeva and others, microbial cells generally carry a negative net charge at the surface due to their membrane proteins, teichoic acids of Gram-positive bacteria, and negatively

charged phospholipids at the outer membrane of Gram-negative bacteria. This way, polycations are attracted and if they have a proportionate amphiphilic character, they are able to disrupt the outer as well as the cytoplasmic membrane and afford lysis of the cell resulting in cell death. So far this has been the only established way of action of polycationic antimicrobial polymers confirmed by numerous studies nicely reviewed by Timofeeva *et al.* [23]. Many examples of such antimicrobial polycations with quarternary ammonium [32] and phosphonium [33,34], tertiary sulfonium [35] and guanidinium functions are known. The first polymer that became a commercial disinfectant was poly(hexamethylene biguanidinium hydrochloride), which was thoroughly investigated by Ikeda *et al.* [36]. The same group presented one of the most potent biocidal polymers against *S. aureus*, a poly(methacrylate) that contains chlorhexidine-like side groups [37].

Closer observation of the known antimicrobial polymeric structures reveals that they are not always polymerized cationic biocides. Numerous biocidal macromolecules that do not contain biocidal repeating units are known to date. The antimicrobial activity of such polymers obviously originates from the whole molecule. Figure 3 shows some examples of polymers that carry a quarternary ammonium group in the main chain or the side chain. In both cases some molecules require biocidal repeating units (left side) in the polymer chain, while others just need a quarternary ammonium group (right side).

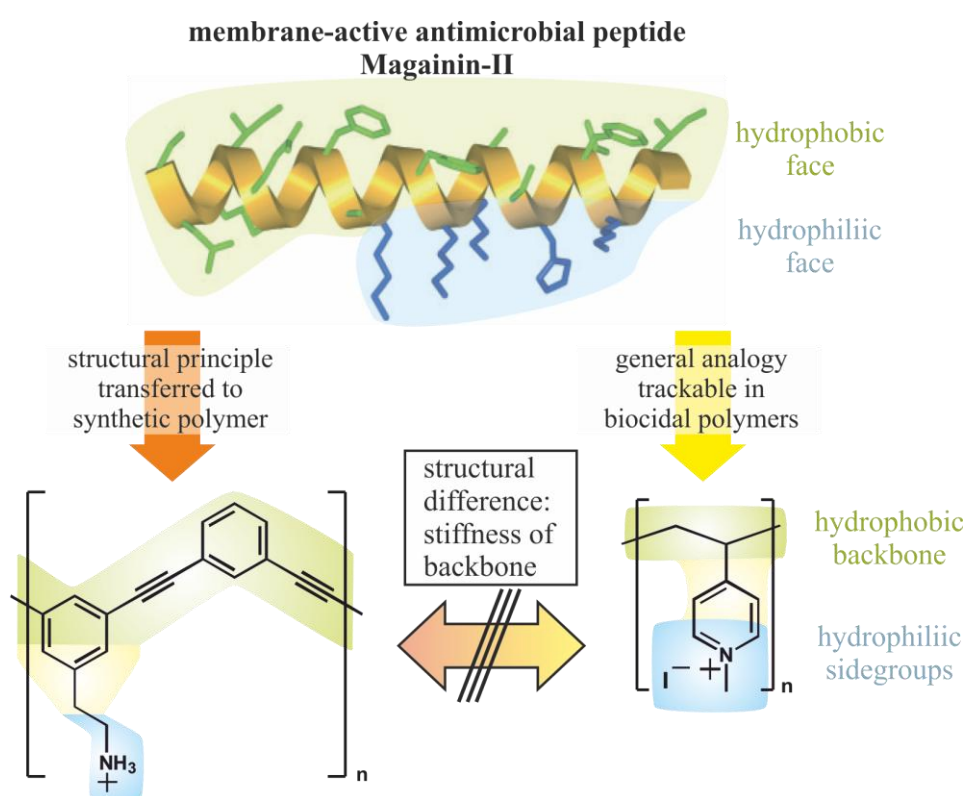
Figure 3. Typical structures of biocidal polymers with quarternary ammonium groups.



It is obvious that biocidal repeating units are not required if the polymer backbone has a hydrophobic character. On the other hand, polymers with a hydrophilic polymer backbone require a hydrophobic region parallel to the backbone, which is provided by the hydrophobic side groups. DeGrado and particularly Tew have intensively and very successfully worked on polymeric mimics for the antimicrobial peptides, such as magainin and defensin [21,38–41]. Such antimicrobial peptides (AMP) are membrane active antimicrobial compounds and their mode of action is based on two basic elements: Firstly, both peptides have a highly rigid backbone and secondly the side groups are organized in a way such that these stiff molecules have one hydrophobic side and one side with a

cationic net charge. Such molecules are highly efficient in disrupting a microbial cell membrane by being inserted with the whole backbone. This intrusion is devastating to the membrane and rips it apart resulting in rapid cell death [42,43]. Poly(phenylene ethynylene)-based conjugated polymers with amino side groups and also other polymers with stiff backbones and cationic side groups are perfect mimics for those peptides and show high antimicrobial activity and low toxicity. Interestingly, also random peptide sequences prepared by ring-opening polymerization of beta-lactams can be tuned to be mimics of antimicrobial peptides [44]. However, taking a look at the known antimicrobial polycations reveals that basically all these polymers could be mimics for magainin (see Figure 4). The only missing element is most often the stiff backbone. Thus, the polycations cannot sufficiently separate the hydrophobic side (backbone or side groups) from cationic groups, which explains the generally lower activity of most biocidal polymers compared to the antimicrobial peptides.

Figure 4. Biocidal polymers as antimicrobial peptide-mimics.



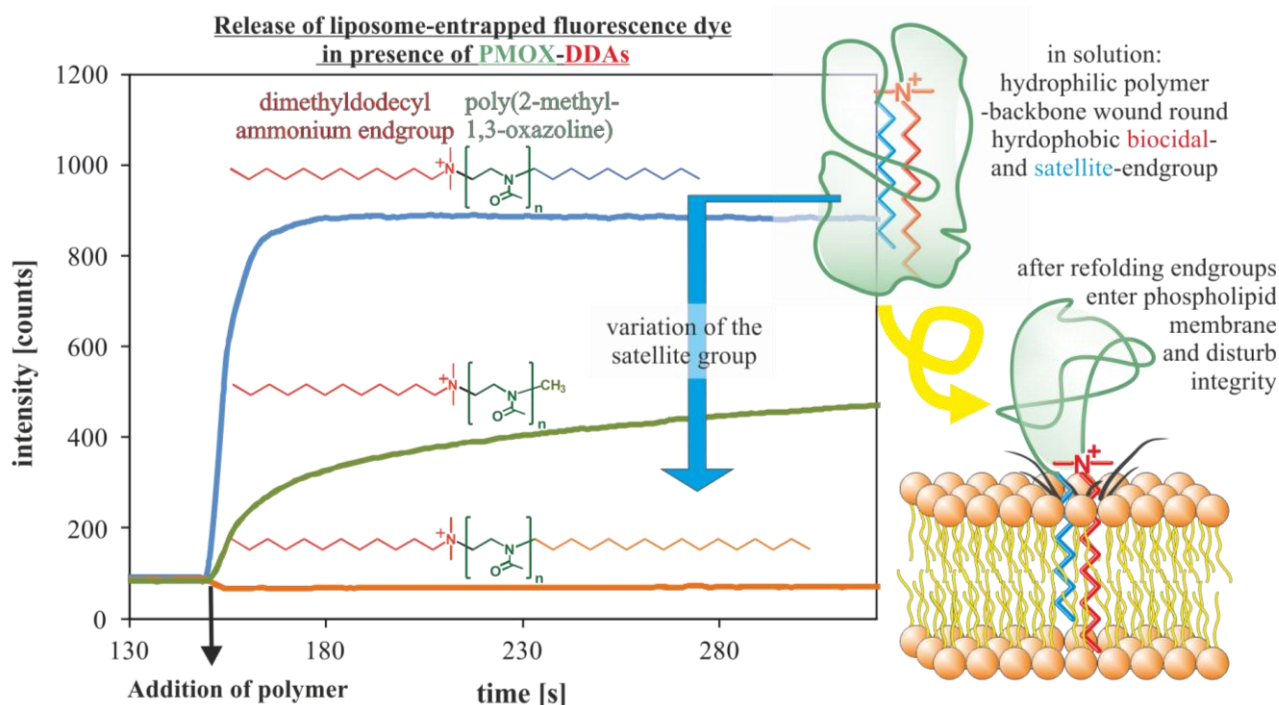
Besides the established quarternary ammonium groups, effective antimicrobial polymers with protonated tertiary and primary amino groups have been described recently. Examples are the above mentioned Poly(phenylene ethynylene)-based polymers. Another example is a random copolymer class of dimethylaminomethyl styrene and octylstyrene, which is antimicrobially active upon protonation of the tertiary amino groups, while the respective quarternized derivatives are less active [45]. Palermo and Kurada synthesized similar copolymers by copolymerizing dimethylaminoethylacrylamide and aminoethylacrylamide, respectively, with n-butylacrylamide and showed that these polymers are antimicrobially active and less toxic to blood cells compared to the respective quarternary ammonium derivatives [46,47]. Another recent development in the group of Timoveeva are poly(diallylammonium salts) that contain either secondary or tertiary amino groups [48]. These polymers show excellent

activity against *S. aureus* and *Candida albicans*. Besides linear polymers, dendritic and hyperbranched polymers have also been described to exhibit strong antimicrobial properties, e.g., quarternized, hyperbranched PEI [49].

A seemingly low molecular weight biocide developed by Das *et al.* might actually work as an antimicrobial polymer, because the molecule is a hydrogelator that forms helical polymeric structures in aqueous solution [50,51]. This might be the reason why this non-toxic molecule shows antimicrobial activity against Gram-positive and Gram-negative bacteria comparable and even superior to antibiotics.

Although polymeric biocides work most effectively when cationic groups are positioned along the polymer backbone, there is another class of antimicrobial polymers that have only one biocidal end group. These polymers are mostly realized by cationic ring-opening polymerization of 2-alkyl-1,3-oxazolines and terminating the macromolecule with a cationic surfactant. One advantage of this polymerization technique is the controlled introduction of a specific group at one end and another group at the distal end of the polymers by choosing a suitable initiator and termination agent. It could be shown that the molecular weight of poly(2-methyl-1,3-oxazolines) (PMOx) and the respective ethyl-derivative (PEtOx) does not influence the activity of the biocidal end group, dimethyldodecylammonium bromide (DDA), in a range of 2,000–10,000 g/mol [29]. Further, a similar derivative containing polyethylene glycol (PEG) showed a 5–10 times lower antimicrobial activity compared to the polyoxazolines. Interestingly, the group distal to the biocide, the so-called satellite group, controls the activity of the whole molecule in a range of 3 orders of magnitude [52]. We propose that this effect is due to the amphipatic behaviour of the macromolecules, *i.e.*, they form unimolecular micelles in solutions and refold at the surface of microbial cell membranes. The satellite group controls micelle formation and insertion of the biocide into the membrane (see Figure 5).

Figure 5. Working concepts of telechelic poly(2-alkyl-1,3-oxazolines) with antimicrobial end group and satellite group. Redrawn according to [52,53].



The proposed mechanism was supported by experiments on liposome/polymer interactions, which showed that the highly active PMOx derivative with an antimicrobial DDA group and a non-bioactive decyl satellite group quickly perforates an *E. coli*-like liposome membrane [53]. When changing the satellite to a hexadecyl group, the whole molecule completely loses its antimicrobial activity, which was again supported by the liposome experiment. It was also demonstrated that the latter inactive macromolecules bind more strongly to the liposomes than the active ones, but without penetrating the membrane. This example illustrates the strong dependence of bioactivity of polymers on structural detail, *i.e.*, the change of 4 methylene groups in a macromolecule with a mass of 10,000 g/mol can dramatically influence the supramolecular structure and its bioactivity. Recently, it was found that the bioactivity of antimicrobial peptide mimics is also strongly influenced by the nature of the end groups [54].

2.3. Biocide-Releasing Polymers

The polymerization of biocide-releasing macromolecules, first achieved by Vogl and Tirell, who polymerized salicylic acid, did not result in polymers with antimicrobial properties [19]. However, they demonstrated that the degradation of the polyester liberates salicylic acid in a controlled fashion. Similar effects were found for acrylate based polymers with salicylic acid side groups [55] and salicylic acid based poly(anhydride esters) [56].

For a long time, tributyltin esters of polyacrylates were believed to be contact-active polymers, because the released TBT kills microbial cells in the surroundings below the detection limit [57].

Worley *et al.* have developed a class of antimicrobial polymers by using N-halamine groups attached to various polymeric backbones [58,59]. The design of these N-halamines allows the long-term storage of active chlorine. This makes these polymers potent oxidants, which in some cases release chlorine or hypochlorite and in the optimized form, as far as the experimental setups allow such insights, they are able to transfer the active chlorine directly to microbial cells killing them by oxidizing the phospholipids of the cell membrane [60].

Recently, another class of biocidal polymers that release NO (nitric oxide) has been introduced by Schoenfisch [61–63]. The polymers contain diazeniumdiolate groups, which can be formed by addition of NO to an amino group containing polymer under great pressure. The optimization of these groups resulted in polymers that release the NO within several hours. However, more work is needed to get to truly long term active antimicrobial polymers.

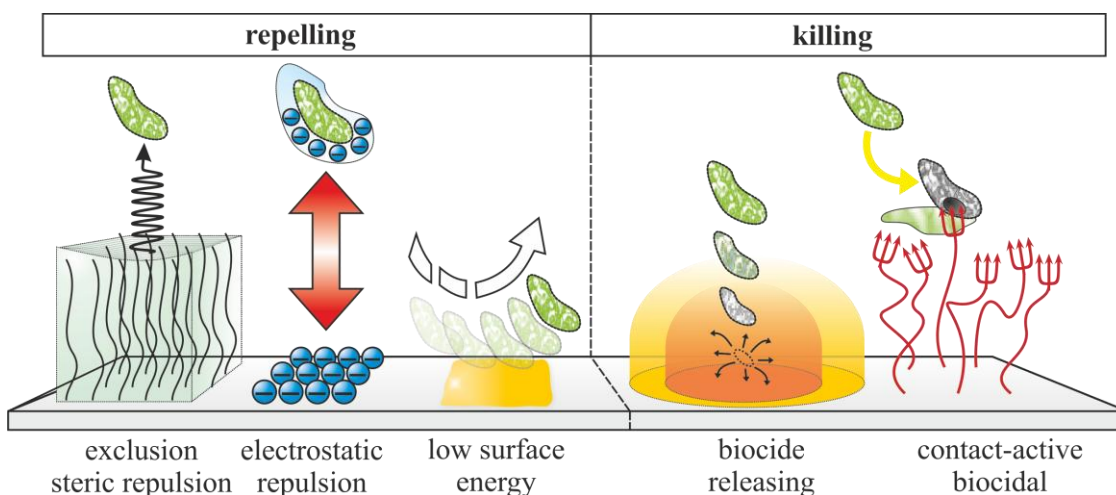
Polyphenols from natural sources such as teas have been found to show antimicrobial properties [64]. Mimics of these natural polymers have been synthesized by Kenawy *et al.* [65]. Investigations on the activity pattern revealed that the polymers have to release phenols in order to kill microbial cells.

A recent intriguing antimicrobial polymer class was introduced by Schanze, Whitten and coworkers, who designed cationic conjugated polyelectrolytes based on poly(phenylene ethynylene)-type conjugated polymers that are functionalized with cationic quaternary ammonium solubilizing groups [66]. These polymers were found to be highly effective in the presence of light by forming and releasing efficiently sensitize singlet oxygen ($^1\text{O}_2$), but they were also effective in the dark, because of their AMP mimicking structure [67]. The singlet oxygen formation also gave the polymer antiviral properties [68].

3. Antimicrobial Surfaces via Attachment of Antimicrobial Polymers

Biofilms on materials are extremely hard to remove and show great resistance to all kinds of biocides. Thus, the prevention of biofilm formation by antimicrobial surfaces is the best way to avoid spreading of diseases and material deterioration. In order to do this, the material must avoid the primary adhesion of living planktonic microbial cells from the surroundings. In general, this can be achieved by either repelling or killing the approaching cells (see Figure 6). Repelling microbes was realized with hydrogel coatings mostly based on PEG or similar hydrogel forming polymers, by highly negatively charged polymers or ultrahydrophobic modifications. The killing of microbes can be achieved by either releasing a biocide from a matrix, which is either previously embedded or actively formed for instance by formation of ROS by photocatalytic TiO_2 . Alternatively, surfaces can be rendered contact-active antimicrobial upon tethering antimicrobial polymers. In the following, only surfaces with attached antimicrobial polymers and multiple working mechanisms will be discussed. Further biocide release systems in general and microbe repelling surfaces are extensively discussed in recent reviews [14,15,21–24].

Figure 6. General principles of antimicrobial surfaces.



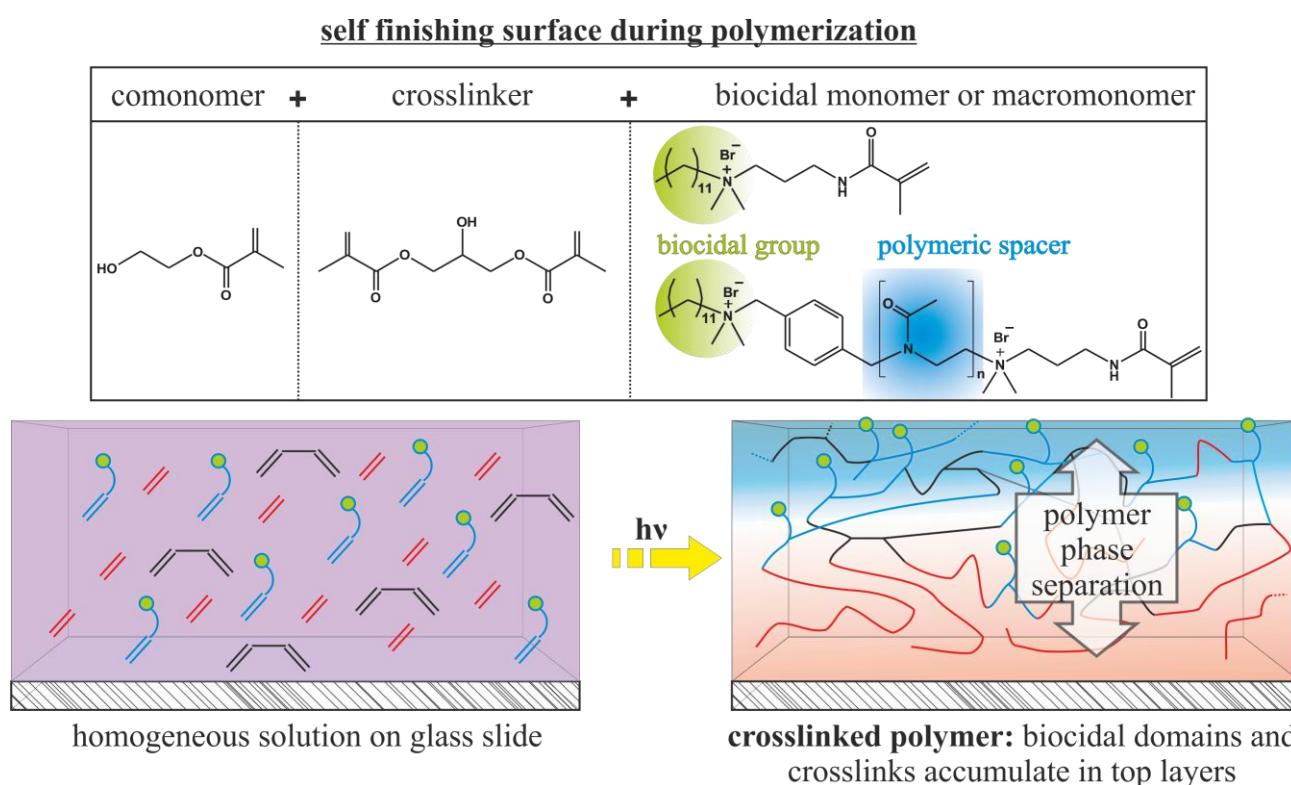
3.1. Techniques towards Surface Attached Polymers

Numerous techniques are known to attach polymers to surfaces, including chemical grafting techniques [69,70], layer-by-layer deposition [71,72] and plasma polymerization [73]. At first, antimicrobial polymers were covalently attached via elaborate techniques. For example, poly-4-vinyl-N-hexylpyridinium was surface-grafted by subsequent modification of a glass slide with an aminosilane and acryloyl chloride. Then the acrylated slide was copolymerized with 4-vinylpyridine, and the product was finally alkylated with hexylbromide [16]. This elaborate procedure required many chemical steps and the use of organic solvents. The side-on attachment of hexylated P4VP to aminoglass was effected by simultaneously reacting the polymer with 1,4-dibromobutane and hexylbromide in the presence of aminoglass [16]. Polyethylenimine (PEI) was also surface-attached to aminoglass via 1,4-dibromobutane, subsequent methylation and alkylation with long chain alkyl halides [74]. Alternatively, the grafting-from method could be used for attaching cationic polyacrylates

to various surfaces, including cellulose filters, via atom transfer radical polymerization (ATRP) of N,N-dimethyl-2-aminoethylacrylamide followed by quarternization [75].

All these elaborate techniques are useful for preparations in the laboratory but not in the industry, because chemically achieved finishing is often too expensive. More recently, surface-attached non-releasing polymers could be realized by incorporating polymeric additives for polyurethane [76,77] as well as acrylate [78] coatings that migrate to the surface of the coating during the preparation process. This way, antimicrobial, contact-active materials can be obtained without a finishing procedure. While the first approach uses 1,3-propylene oxide soft blocks with alkylammonium groups as crosslinked soft segments, the second approach applies macromonomers based on polyoxazolines with biocidal quarternary ammonium end groups [29,52,53], which are copolymerized with the acrylate monomers and crosslinkers (see Figure 7).

Figure 7. Surface-grafting of biocidal polymers via self-induced migration to the surface. Created according to [78].



Besides chemical grafting it is often convenient to coat surfaces with antimicrobial polymers, e.g., by Layer-by-Layer (LbL)-techniques [79]. Also coatings with water-insoluble block copolymers or comb-like block copolymers containing an antimicrobial block, or with water-insoluble quarternized PEIs all from solutions in organic solvents have resulted in long-lasting antimicrobial coatings [80]. In order to avoid organic solvents, block copolymers with a hydrophobic and a hydrophilic, antimicrobial block were used as emulsifiers for the emulsion copolymerization of styrene and acrylates. The resulting paint-like coating can be derived from aqueous suspensions [81].

Another way of coating is the surface attachment of antimicrobial hydrogels. Several examples of gelling antimicrobial peptides, a Pyrene-derivative of Vancomycin and biocidal cationic surfactants

have been reported [51,82]. These compounds form hydrogels from their aqueous solutions. The structure of the gels suggests that the compounds form polymers first and those build up networks incorporating the water. The concept of surface-induced hydrogelation [83,84] allows such compounds to gel on surfaces from their diluted aqueous solution. Even compounds that do not form hydrogels have been reported to gel on an affine surface [85]. Another contact-active surface is a hydrogel formed by a cationic hair-pin like antimicrobial peptide that is also a hydrogelator [86].

The techniques for obtaining contact-active antimicrobial surfaces and representative examples are summarized in Table 1.

Table 1. Examples of surface attached biocidal polymers.

	Method	Polymer	Ref.
Grafting from	Immobilized initiator	QPAM	[75]
		PEtOx	[87]
Grafting to	Immobilized comonomer	QP4VP	[16]
		AMP	[88,89]
		QPEI	[90]
		NB	[91]
	Parallel grafting to and modification	QP4VP	[17]
		PMOx	[78]
		QPU	[76]
		PMOx	[78]
Coating	Layer-by-layer	Polylysine	[79]
		PAA	[92]
		PHGH	[93]
		Chitosan	[94]
	Particles with grafted polymer	Magnetide with QPEI	[95]
		PA-particles with QP4VP	[81]
	Hyperbranched Polymers	QPEI	[80,96]
	Plasma Polymerization	PDAA	[97]
		Polyterpenol	[98]
	Surface induced Hydrogelation	Vancomycin	[82]
		AMP	[86]

Abbreviations: QP4VP: Quarternized poly(4-vinylpyridine); QPAM: Quarternized poly(N,N-dimethylaminoethylacrylamide); PAA: poly(allylammonium chloride); QPEI: Quarternized polyethyleneimine; PS: poly(styrene); PEtOx: poly(2-ethyloxazoline); PMOx: poly(2-methyloxazoline); QPU: Quarternized polyurethanes; PN: Norbornene-based polymers; AMP: antimicrobial peptides; PA: poly(acrylate); PHGH: poly(hexamethylene guanidinium hydrochloride).

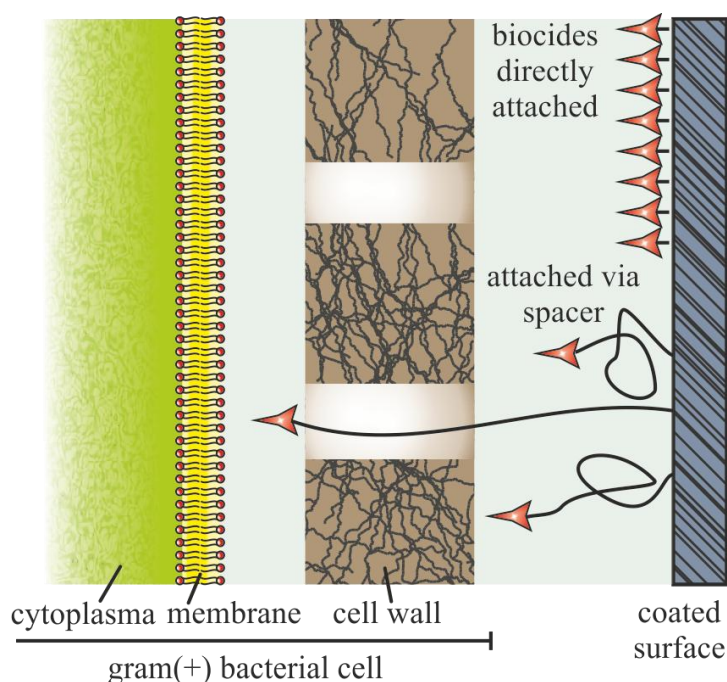
3.2. Functional Principles of Contact-Active Antimicrobial Polymers

As discussed above, the attachment of polymeric biocides or biocidal non-releasing polymers can result in surfaces that kill microbial cells on contact without releasing a biocide. Although, the claim has been made by many, it is rather difficult to prove. Common tests for antimicrobial activity cannot distinguish between contact-activity and release systems. The reason for this is that a biocide on or in a material will diffuse out of the material if it is not covalently attached. However, in order to be lethal for microbes, the concentration has to be above the minimal inhibitory concentration (MIC) or even

the minimal biocidal concentration (MBC) only in a region of one μm above the surface. If the biocide concentration is below MIC outside the small kill zone, all existing tests conclude contact-activity. So far there is no test that can distinguish between those cases. The only way to get a better picture is to perform long-term washing tests and constantly monitor and concentrate the washing solutions as well as testing the antimicrobial performance.

One of the first models of how surface attached antimicrobial polymers might act as a contact-active surface was proposed in 2001 (see Figure 8) [16].

Figure 8. Concept of contact-killing via the polymeric spacer effect. Created according to [16].



The concept of the polymeric spacer effect presumes that a surface grafted biocidal polymer might be capable of penetrating the bacterial cell wall of an adhered bacterial cell. If it reaches the cytoplasmic membrane the cell can be killed by disruption of the phospholipid bilayer. Further, surface grafted polymers are presented in such a high concentration to an adhering cell that they might even kill microbes that are not very susceptible to those polymers in solution. This killing efficiency of surface grafted N-hexylated P4VP could be shown for Gram-positive *Staphylococci* as well as Gram-negative *Pseudomonas aeruginosa*. Remarkably, the latter microbial cells cannot be killed by the polymer in solution, which thus supports the high surface concentration hypothesis. It further indicates that the antimicrobial surface-bound polymer works differently to the polymer in solution. Interestingly, there is no significant difference between non-resistant *S. aureus* cells and MRSA that contain a particularly high numbers of proton pumps, which obviously cannot work at a surface with a grafted antimicrobial polymer [99]. Quarternized surface grafted polyethyleneimine, which effectively kills microbes [74] and even deactivates certain influenza viruses [90] shows a dependence on the molecular weight of the polymer. As calculations indicate, the polymer must be sufficiently large to work efficiently. This strongly supports the polymeric spacer effect. Subsequent work showed that surface grafted quarternized PEI did not build up resistant *S. aureus* or *E. coli* strains [4]. Coatings

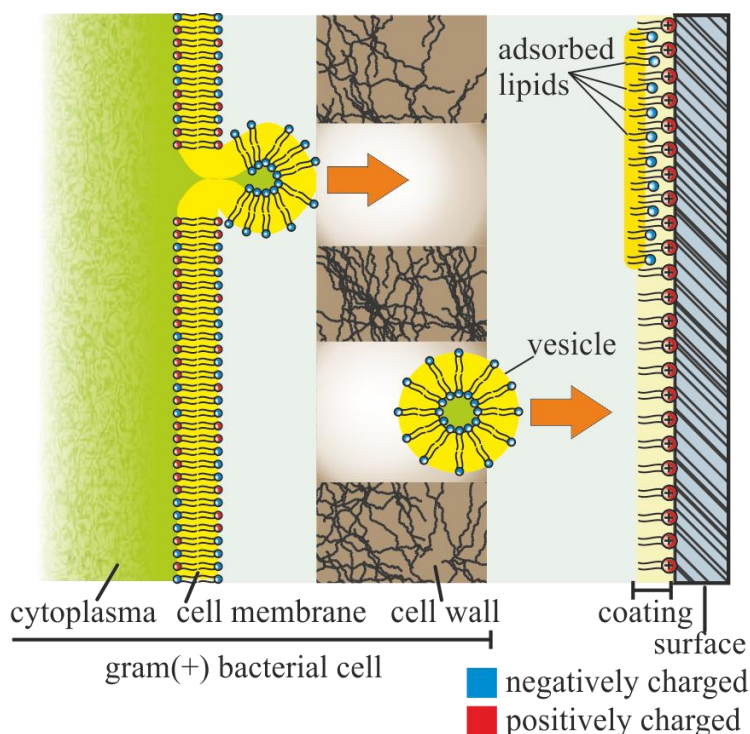
based on single-walled carbon nanotubes were claimed to be antimicrobially active, because the nanotubes would poke through the cell walls of approaching microbial cells, *i.e.*, they also work according to the polymeric spacer effect [100]. As shown by Dathe *et al.* who immobilized various cationic AMPs on Penta Gels [88] and others who grafted the well-known membrane active AMP magainin I via a polymeric spacer using ATRP [101], the concept of the polymeric spacer seems to be valid for peptides as well. On the other hand, the immobilization of magainin I without a polymeric spacer as SAM on gold showed reduced bacterial adherence and killed adhered bacteria as well although biofilm formation could only be delayed [102].

Another recently published work has explored comb-like polymer coatings, where an antimicrobial DDA-group is grafted to cellulose via a PEtOx of spacer varying in length [87]. Surprisingly, all coatings, even those without a polymeric spacer, were active against *S. aureus* cells. When treating these coatings with negatively charged phospholipids in order to simulate contact with a microbial cell membrane, all coatings adsorbed similar amounts of the phospholipid but only the coatings with polymeric spacers of less than some 90 repeating unit of the PEtOx were no longer antimicrobially active after this treatment. This indicates that there might be more than one functional principle of such antimicrobial surfaces.

In order to gain insights into a second possible mechanism, a series of coatings of cellulose derivatives with different quarternary ammonium groups and additional hydrophobic groups has been investigated [103]. The result of the antimicrobial assessment showed that a surface can kill *S. aureus* cells mainly controlled by the cationic/hydrophobic balance and not so much by the charge density. Treatment with negatively charged phospholipids did quench the antimicrobial activity of all investigated surfaces as well as that of the well known antimicrobial surface derived by coating surfaces with the silane 3-(trimethoxysilyl)-propyldimethyloctadecylammonium chloride referred to as DOW 5700 [104]. From these results, it was proposed that the antimicrobial action of coatings that are not modified with biocidal functions on long polymeric spacers originates from their ability to adsorb negatively charged phospholipids (see Figure 9) [103]. Removal of such molecules from cell membranes might indeed lead to their disruption. The weak point of the proposed phospholipid sponge effect is that it cannot explain how the water-insoluble phospholipids cross the cell wall and reach the antimicrobial surface. One possible hypothesis is that the phospholipids travel as liposomes through larger holes in the microbial cell wall.

The increasing number of contact-active antimicrobial surfaces with short chain antimicrobial polymers or just surface attached biocidal groups has resulted in some other models for the functional principle of antimicrobial surfaces [91,105,106]. Kugler *et al.* have grafted P-4VP to glass surfaces and investigated the influence of the degree of quarternization on the antimicrobial action [107]. The very sharp transition between the active and inactive state at a minimal degree of alkylation was interpreted in terms of a mechanism that somehow involves the exchange of divalent ions of the cell wall. Other authors have followed this unlikely scenario [108,109]. Another proposed mechanism suggests that the hydrophobic surface and the negative surface net charge of microbes leads to an attraction of the cells to cationic surfaces, which has been shown in many scenarios, and somehow destroys their cell envelop [110].

Figure 9. Concept of contact-killing via the phospholipid sponge effect. Created according to [103].



An alternative way to create contact-active surfaces is to immobilize antimicrobial proteins. The first enzyme tested for the attachment to surfaces was the mureinase lysozyme, which was first described as an antimicrobial protein by Fleming in 1922 [111]. Although most approaches to lysozyme-immobilization have resulted in complete loss of its cell wall-lytic properties, some alternative ways to immobilize lysozyme, including polymeric spacers, have led to surfaces capable of killing *Micrococcus lysodeikticus* and *Bacillus subtilis*, and growth-inhibiting or even killing *S. aureus* [112–116].

Recently, more effective antimicrobial enzymes, such as the endopeptidase lysostaphin [117], enzymes with mureinase and endopeptidase activity [118], lysins [119] and the fast killing, pneumococcal bacteriophage lytic enzyme (Pal) [120] have been found.

Dordick *et al.* published a coating based on lysostaphin, immobilized on carbon nanotubes [121]. The coating is long-term active and efficiently kills MRSA on contact. It is conceivable that the carbon nanotubes stick out of the surface and in this way reach deep into the microbial cell wall.

4. Multifunctional Antimicrobial Surfaces

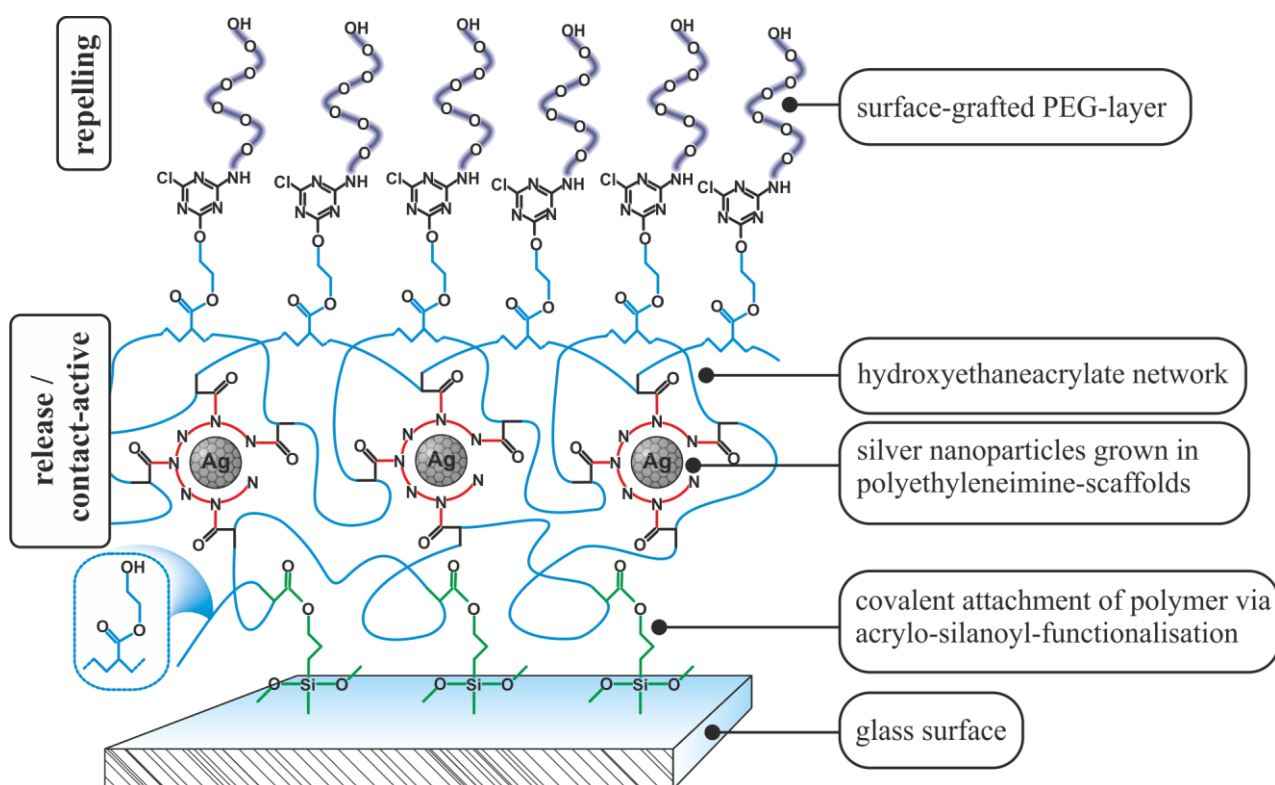
4.1. Repelling and Releasing

Coatings based on biocide-releasing polymers often change their surface properties after liberating the biocide. If the resulting polymers are water-soluble or at least hydrophilic, the surface can repel the attached microbe corpses and hereby refresh the material. The most prominent examples are antifouling paints. They combine the release of a biocide with frequent renewing of the surface (self-polishing), which washes away all attached microbes and other marine organisms dead or alive. Such coatings work most effectively on ship's hulls, because the shear of the moving ship strongly

supports the self-polishing. Modern antifouling paints are coatings of copper ion-releasing, strongly hydrophobic organo copper esters of poly(methacrylic acid) copolymers. The most important aspect is that the hydrophobic coating does not swell in water. Thus, only organo copper ester groups in the upper 10–100 nm are hydrolyzed. The resulting hydrophilic poly(methacrylate) is removed by shear and a fresh contamination free surface is revealed. Unfortunately, the relatively harmless copper is not sufficient to kill all attached marine organisms and thus the coatings are filled with so-called booster biocides, which are usually pesticides [122]. Although these antifouling paints release large amounts of biocides they are only 80 to 90% as effective as the Tributyltin-based coatings which were banned in 2003 [123]. Alternative benign but still effective antifouling coatings, for instance paints with natural biocides instead of the synthetic pesticides [124] are not yet on the market.

Besides antifouling paints, several approaches can be considered as release and repel systems. For instance typical hydrogel coatings for implants usually based on polymers such as polyvinylalcohol, PEG-copolymers or poly acrylic acid derivatives, tend to reduce the microbial adhesion by nearly two orders of magnitude compared to the uncoated control. Loading these hydrogels with antibiotics or other biocides, makes these coatings more active and in principle capable of simultaneously repelling and releasing systems. A more complex design described is a coating based on a hydrophilic polymer network of poly(2-hydroxyethylacrylate) with PEI crosslinking points (see Figure 10). The latter are capable of selectively taking up silver ions from aqueous solution and act as a template for the formation of silver nanoparticles [125]. PEGylation of these conetworks results in materials that efficiently kill *S. aureus* cells and still repel them after exhaustion of the silver.

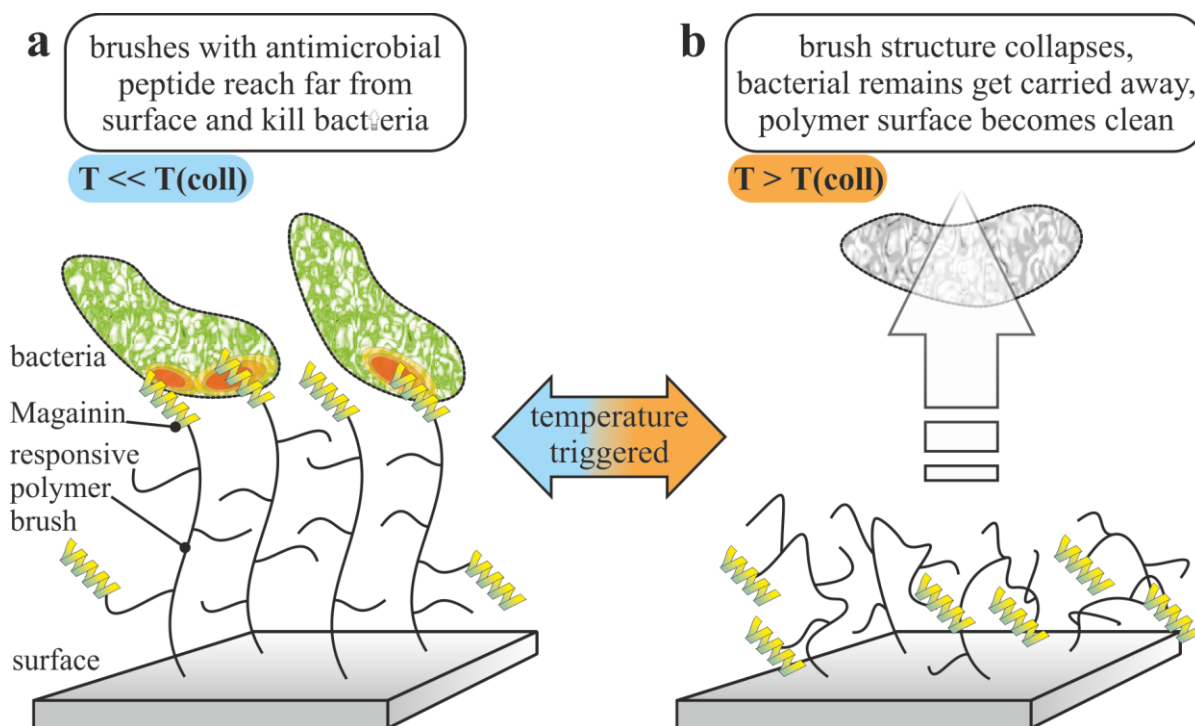
Figure 10. Concept of repel and release of a designed network. Created according to [125].



4.2. Contact-Killing and Repelling

Contact-active surfaces are often considered to be self-deactivating, because killed microbes may adhere to the active layer and new approaching microbes can adhere and proliferate on these corpses. An improvement to such surfaces would be a material that kills microbes and repels the dead cells simultaneously. Chen *et al.* have created an antimicrobial contact-active surface against *E. coli* by attaching poly(N,N-dimethyl-N-(ethoxycarbonylmethyl)-N-[2'-(methacryloyloxy)ethyl]-ammonium bromide) via ATRP [126]. The constant contact with the aqueous environment results in hydrolytic cleaving of the ethyl ester next to the quarternary ammonium head group. The resulting zwitterionic head group is no longer biocidal, but effectively repels all attached microbial cells, dead or alive. This interesting effect is not yet reversible, but impressively shows the opportunities of such surfaces. More recently Laloyaux *et al.* have presented a surface that consists of surface attached magainin grafted with oligo(ethylene glycol) methacrylates [127]. The polymer chains are stretched at room temperature and effectively kill microbial cells. When heated over 35 °C the polymers collapse representing the PEG at the surface, which repels the attached and non-attached Gram-positive cells efficiently. By lowering the temperature, the killing properties are reactivated. In principle, this allows to kill or repel microbial cells by frequent temperature sweeps (see Figure 11).

Figure 11. Concept thermoresponsive surfaces with magainin grafted via thermoresponsive oligo(ethylene glycol) methacrylates that (a) kill bacterial cells below and (b) repel them above the transition temperature. Created according to [127].



4.3. Releasing and Contact-Killing

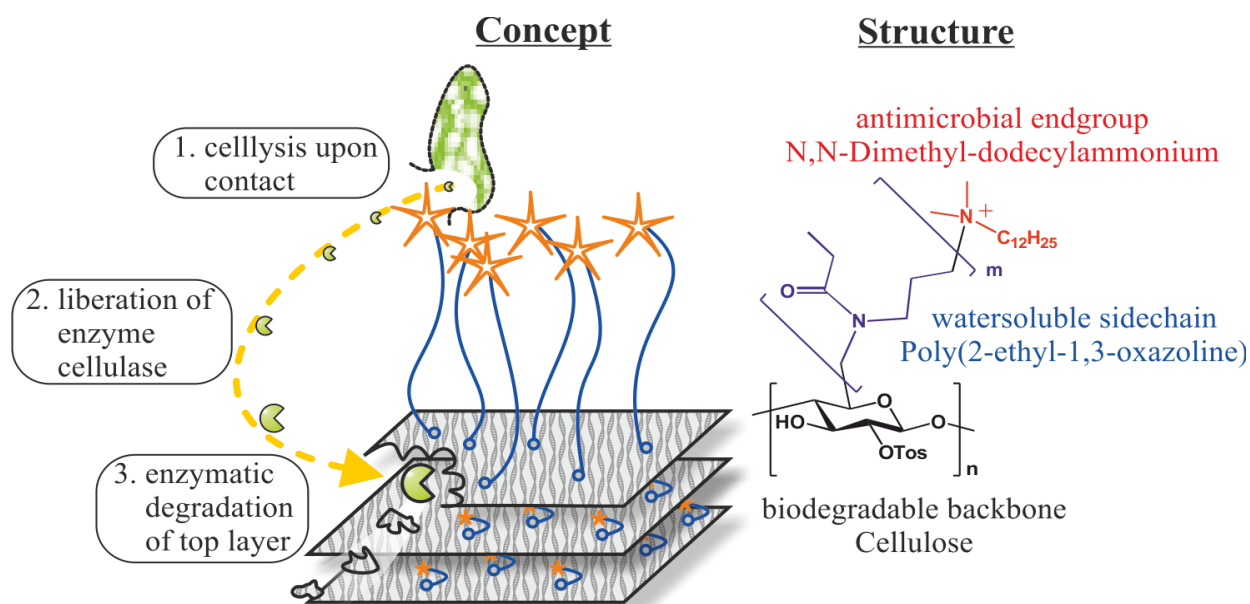
Another way to prolong the activity of an antimicrobial release system is to combine this concept with a contact-active killing approach. Worley and coworkers have pioneered the release of oxidative

chlorine compounds as slow release and even as release on demand systems [128]. By combining the above described halamine-containing groups with biocidal quarternary ammonium groups in a polyurethane coating, the antimicrobial effect caused by the release of hypochlorite was significantly prolonged by the additional non-releasing antimicrobial groups, which continue to kill the microbes even after the release system is exhausted [129]. A similar behaviour could be afforded by the incorporation of silver nanoparticles into a LbL coating and finishing it with quaternary ammonium groups [130]. The release of silver ions is accompanied by the contact-killing of the layer that contains quaternary ammonium groups. Another benefit of this coating is the successfully demonstrated reloading with silver nanoparticles using silver salts. The same is hold true for the halamine-coatings as demonstrated by Liang *et al.* [129].

As demonstrated by Shamby *et al.*, poly(4-vinylpyridine) alkylated with hexylbromide reacts with silver nitrate in aqueous solution to form water-insoluble conjugates of the antimicrobial polymer and silver bromide nanoparticles. Coatings of these composites simultaneously kill microbes by releasing silver ions and upon contact with the quarternary ammonium groups [131]. Gyomard *et al.* claim that the natural antimicrobial peptide gramicidin A immobilized into a LbL matrix kills *E. faecalis* cells in the surroundings upon release and in the surface-immobilized form [79].

More recently, we have developed a coating based on cellulose with an antimicrobial DDA group grafted via PETox, which kills approaching microbial cells on contact [87]. Once a microbial cell is disrupted on the surface, the cell liberates the ubiquitous enzyme cellulase. We could show that this enzyme is capable of degrading the coating and reactivating it in a way similar to the above described release on demand systems (see Figure 12). In contrast to common self-polishing antifouling paints, the cellulose-based coating acts as a contact-active system, is biologically compatible, degradable, and additionally might release biocides in case of a biological contamination only.

Figure 12. Concept of a cellulose-based coating with an attached biocidal polymer that can be degraded by cellulase. Created according to [87].



5. Conclusions

The present manuscript considers the major functional principles of antimicrobial polymers in solution and on surfaces. Most cationic antimicrobial polymers seem to work in a way similar to membrane-active antimicrobial peptides, such as magainin. Alternative polymers get their biological activity from either presenting biocidal side groups that act the same as in their free form or the polymers have to release biocidal groups. In general, antimicrobial polymers can be classified as polymeric biocides, biocidal polymers, and biocide-releasing polymers. The attachment of the second class results in contact-active antimicrobial surfaces. However, the functional principle of such surfaces is controversially discussed in the literature. So far, it seems that there are at least two mechanisms, the polymeric spacer effect and the phospholipids-sponge effect. However, other possible mechanisms cannot be excluded and a significant amount of work has to be done in order to clarify the proposed mechanisms. For future developments of antimicrobial surfaces, it seems desirable to combine different working mechanisms and to optimize release on demand systems.

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