Kill&Repel Coatings: The Marriage of Antifouling and Bactericidal Properties to Mitigate and Treat Wound Infections

Manuela Garay-Sarmiento, Lena Witzdam, Mariia Vorobii, Christian Simons, Niklas Herrmann, Andres de los Santos Pereira, Elisabeth Heine, Islam El-Awaad, Rudolf Lütticken, Felix Jakob, Ulrich Schwaneberg,* and Cesar Rodriguez-Emmenegger*

Wound infections originate when exogenous or endogenous bacterial pathogens can circumvent the barrier of the wound dressing and invade the wound bed. Bacterial colonization causes inflammation, stalls the healing process, and carries the risk of dissemination to other tissues. In addition, current antimicrobial dressings fail to resolve an infection once it has been established because debris of the killed bacteria rapidly accumulates on their surface and hampers the antimicrobial action. Faced with this challenge, hybrid synthetic-natural water-soluble macromolecules are designed that self-assemble onto the surface of dressings to generate an antifouling brush functionalized with endolysin, a bactericidal enzyme that poses no harm for eukaryotic cells. The simultaneous action of the brush and the enzyme not only prevents the colonization of the dressing, but also enables the coating to kill planktonic bacteria with even higher efficiency than the free enzyme. Remarkably, the Kill&Repel coating could completely eradicate bacteria in a simulated infection without allowing the adhesion of residues on the surface. Thus, this strategy opens a revolutionary approach for protecting and treating an infected wound in a safer and more efficient manner.

1. Introduction

Wound dressings are an artificial barrier that protect a wound from exterior agents. Unfortunately, bacteria can often violate this barrier as well as the natural ones leading to serious infections. Even the most advanced bactericidal dressings cannot fully protect the wound as debris from killed bacteria adheres, thus blocking the active properties. To address this fundamental challenge, we introduce a Kill&Repel ultra-thin coating that simultaneously kills bacteria and prevents the adhesion of their debris providing a superior protection to the wound.

Healing of a wound requires a finely regulated sequence of events in multicellular organisms. It progresses through the inflammation phase, the proliferation phase and the remodeling phase. Once the skin is injured, microorganisms such as bacteria are able to infiltrate the underlying tissue causing delayed wound healing and infections. Furthermore, contamination of the wound with bacteria causes an elevated secretion of pro-inflammatory cytokines, which prolongs the inflammatory phase even to the extent of generating a chronic wound if bacteria are not cleared. Chronic wounds can remain in the inflammatory phase for weeks or even years without healing progress.1 They require prolonged care at the risk of developing multiple infections. Until now, the treatment of skin wounds and the prevention of bacterial infections remains a significant medical and socio-economic challenge in our society. In 2014, the US health insurance system Medicare estimated the annual cost for treatment of infected and chronic wounds to range from $28.1 to $96.8 billion.2 Therefore, the rapid development of advanced wound dressings capable of minimizing the development of infections and chronic conditions is imperative.

Current mainstream technologies for antimicrobial wound dressings rely on two strategies: 1) the release of bactericidal compounds such as silver ions (Ag+) or antibiotics and 2) coatings based on polycations, which exploit the electrostatic attraction between cationic polymers and the negatively charged bacteria to disrupt or even tear the cell membrane. However, these systems have also been shown to harm human cells and therefore can only provide limited support for a prolonged time.3 Silver ions in wound dressings were shown to...
decrease cell viability, collagen synthesis, cell proliferation, and even alter cell morphology of diabetic fibroblasts. Antibiotics can compromise the innate immune system of the patient and the lack of specificity can cause the selection of resistant strains. Coatings based on polycations exert strong forces that can disrupt the cell membrane of bacteria causing their death. However, the same physical principle also causes toxicity to eukaryotic cells. These adverse effects are detrimental to the healing of wounds. Moreover, all of these systems fail to provide an antifouling interface. The deposition of proteins, cells, and bacterial debris on top of the coating drastically reduces their bactericidal activity and ability to prevent bacterial adhesion. This creates conditions that support further bacterial colonization with subsequent biofilm formation. The minimization of interactions combined with bactericidal activity is a sine qua non condition for an advanced wound dressing.

The ideal wound dressing should comprise an antimicrobial mechanism, which not only is very fast and effective but also does not induce any resistance. It must combine repelency (antifouling) with an active mechanism to kill bacteria if these are able to invade or are already present in the wound bed (infected wounds). Antifouling properties are important to avoid the uncontrolled adhesion of cells and debris to the wound dressing, which would ultimately end up blocking the active surface. In addition, adhesion of skin cells, immune cells, and bacteria will lead to the release of excessive amounts of pro-inflammatory cytokines, which exacerbates inflammation and thereby impairs the healing process. Antifouling dressings are also essential to prevent conglutination of the dressing to the wound and consequent damage of tissue when the dressing is exchanged.

To date, the best antifouling system to prevent protein adsorption is hydrophilic polymer brushes grafted from a surface at a very high density. They provide enthalpic and entropic barriers, which prevent both penetration and direct adsorption of proteins. Polymer brushes from zwitterionic carboxybetaines, and N-(2-hydroxypropyl)methacrylamide (HPMA) have superior resistance to protein adsorption from bodily fluids such as saliva, urine, cerebrospinal fluid, and even human blood plasma. Moreover, the introduced repellency extends to the mesoscopic scale minimizing the attractive forces to bacterial adhesion as measured by single-cell force spectroscopy. Effective polymer brushes demand effective ways to link the initiators and to be able to polymerize from the surface of the dressing. Linking the initiator group can be challenging due to the common low chemical reactivity of the polymers of dressings. Current strategies require harsh treatments such as oxidation with plasma and subsequent functionalization with silane initiators. However, limited stability of the brush tethering siloxane bond under aqueous conditions leads to partial detachment of the polymer brush (delamination). The challenge is further aggravated by the rather stringent conditions for surface-initiated controlled polymerization of the brushes. These challenges have so far precluded the translation of polymer brushes to real applications. Recently, our group synthesized protein–polymer hybrids, which enable the rapid modification of different kinds of materials and surfaces without pretreatment. Some of these surfaces are polymers, metals, and natural-origin including polyesters, polystyrene, silicones, cyclic olefin copolymer, gold, dental enamel, hair, and plant leaves.

The hybrids consist of a surface affine fusion protein (SAP) and an antifouling polymer block. Both parts are hydrophilic and molecularly dissolved in water. In the presence of a surface, the protein changes its molecular conformation to maximize weak interactions and lower the interfacial energy, resulting in its irreversible adsorption while segregating the polymeric part of the molecule away from the surface, leading to an oriented type of immobilization. This oriented adsorption of the hybrids by the protein leaves a brush-like coating on the periphery of the surface that exhibits antifouling properties on par with the best polymer brushes but lacks bactericidal properties.

Herein, we designed an ultrathin coating for wound dressings, which we termed the Kill&Repel modification. We combined the outstanding performance of polymer brushes with the rapid killing effect of endolysins to build a hybrid coating that renders the surface of wound dressings with excellent and effective antifouling and bactericidal properties. The coating consists of our previously developed antifouling hybrids (LCI-eGFP-Polymer) and newly developed bactericidal hybrids (LCI-EndLys). An oriented type of immobilization is essential to avoid impairment of enzyme activity (Figure 1). Endolysins are an emerging class of bactericidal enzymes, which may provide a solution to antibiotic resistance. Bacteriophages produce them at the end of their reproduction cycle to cleave the peptidoglycan layer at specific sites resulting in the death of bacteria and escape of progeny phages. The evolutionary selection of these enzymes makes them orders of magnitude less likely to generate resistant strains. Moreover, they are completely innocuous for eukaryotic cells. In the present work, we chose PlyGBS94 (hereafter EndLys) as a model endolysin, which originates from a Streptococcus agalactiae phage. EndLys is a mutant lysin with enhanced killing activity containing only the endopeptidase domain from the N-terminal region of native PlyGBS. It targets, among others, Streptococcus agalactiae, an important Gram-positive pathogen and major cause of neonate meningitis and sepsis. Lately, it has been increasingly associated with severe subcutaneous and soft tissue infections as well as chronic wounds in adults. Moreover, an increase in antibiotic resistance of these bacteria has recently attracted attention, thus an alternative strategy to combat this pathogen is needed.

We utilized flat surfaces and electrospun meshes of poly(e-caprolactone) (PCL) as model substrates and demonstrated by surface plasmon resonance (SPR) and in vitro experiments that LCI-eGFP-Polymer could prohibit the nonspecific adsorption of proteins, the adhesion of skin cells as well as biofilm-forming bacteria. We formed the Kill&Repel coating by the co-adsorption of LCI-eGFP-HPMA and LCI-EndLys on the surface at a ratio of 90 to 10 mol%, respectively. We confirmed that the coating did not exert any cytotoxic effect and was harmless to human cells. Using SPR, we proved that the co-immobilization with LCI-EndLys did not hamper the antifouling properties against proteins from blood plasma previously achieved by LCI-eGFP-HPMA. Moreover, the Kill&Repel coating was capable of preventing the nonspecific adhesion of dermal
fibroblasts to the meshes. By means of a fluorogenic substrate, we elucidated that the adsorption process and combination with LCI-eGFP-pHPMA did not affect the enzymatic activity of LCI-EndLys; on the contrary, the proximity to pHPMA boosted the enzymatic and bactericidal activity of EndLys, enabling the Kill&Repel coating to effectively scavenge planktonic bacteria, clear biofilm on simulated infected wounds and simultaneously protect the dressing surface from the accumulation of debris.

2. Results and Discussion

Protein adsorption occurs within milliseconds of biomaterials being exposed to bodily fluids—so why not utilize the adhesion advantage of proteins to solve the long-standing problem of how to bring biofunctionality to technical surfaces from which conventional modification methods cannot be performed? In this work, we developed a non-adhesive and antimicrobial coating for wound dressings based on this principle. The molecular adsorption of hybrid molecules to the surface of the dressings forms an ultra-thin and continuous coating at the periphery. The surface affine block of these hybrids consists of the antimicrobial peptide liquid-chromatography-peak-I (LCI) and the enhanced green fluorescent protein (eGFP). The LCI-eGFP fusion protein was produced in Escherichia coli BL21 Gold (DE3) cells as previously described by our group.\[14a,c\] Antifouling protein-polymer hybrids were synthesized by conjugating the maleimide initiator, 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl) ethyl 2-bromo-2-methylpropanoate, via thiol-Michael click reaction to the one available cysteine residue of eGFP (position 69). Therefrom, (3-methacryloylamino-propyl)-(2-carboxy-ethyl) dimethylammonium carboxbetaine methacrylamide (CBMAA) and HPMA chains were grafted via SET-LRP (Figure 2a). The synthesis of LCI-eGFP-pCBMAA and LCI-eGFP-pHPMA hybrids was carried out in PBS buffer at 30 °C for 18-20 h, yielding conversions of 66–86% as determined by proton nuclear magnetic resonance spectroscopy (1H NMR) (Table S1 and Figure S1, Supporting Information). After purification with Amicon Ultra centrifugal filters, the molecular weight was determined by multiangle laser light scattering size exclusion chromatography (MALLS-SEC) in sodium acetate buffer (Table S1, Supporting Information). MALLS-SEC analysis showed narrow unimodal molecular weight distributions indicating quantitative initiation efficiency and a controlled polymerization process, i.e., a polydispersity of 1.33 for the poly(HPMA) and 1.27 for poly(CBMAA) hybrids (Figure 2b). Moreover, the obtained molecular weights (M_n,SEC) are in good agreement with the theoretical molecular weights calculated from 1H NMR (M_n,conversion), further supporting a quantitative initiator efficiency.

The formation of an antifouling coating on electrospun PCL dressings was carried out by simply immersing the dressing in the purified LCI-eGFP-Polymer solution at a concentration of 1 mg mL\(^{-1}\) in PBS. Incubation proceeded for 1 h at 4 °C followed by rinsing with copious amounts of PBS buffer and ultra-pure water. By x-ray photoelectron spectroscopy (XPS), we confirmed the successful modification of PCL dressings with LCI-eGFP-pHPMA and LCI-eGFP-pCBMAA. The high-resolution spectrum for C1s of the coated fibers shows signals of C= and N= at 285.5 and 287.9 eV (Figure S2, Supporting Information). These signals correspond to the amide bonds of pristine PCL fibers. Moreover, the N1s spectra of both polymers show characteristic signals for amide bonds (400 eV) and for the quaternary ammonium (402.6 eV) of poly(CBMAA) (Figure 2c). The binding of the LCI-eGFP-Polymer hybrids was quantified by SPR. For these studies, a thin film of PCL was spin-coated on gold-coated SPR sensor chips to mimic the
Figure 2. a) Synthetic route to polymerize LCI-eGFP-Polymer hybrids. b) Elution plots of LCI-eGFP in comparison to the polymerized hybrids by MALLS-SEC. Solid lines display the normalized refractive index change over elution time. Dashed lines refer to the corresponding molecular weight. c) High resolution N1s XPS spectra of (i) bare PCL mesh, PCL coated with (ii) LCI-eGFP, (iii) LCI-eGFP-pCBMAA, (iv) LCI-eGFP-pHPMA, LCI-eGFP-pHPMA after incubation for 180 d in (v) PBS and (vi) LB supplemented with 10% FBS. All spectra were normalized to maximum intensity. d) CLSM images of PCL meshes coated with LCI-eGFP-HPMA and stability studies in PBS and LB + FBS: (i) before coating, (ii) immediately after coating, (iii) after 24 h incubation and (iv) after incubation for 32 d. Scale bars are 50 µm.
surface of PCL nanofibers. The thickness of the PCL film was determined to be 17 nm by ellipsometry. The LCI-eGFP-polymer coatings were formed inside the flow cell of the SPR by injecting the different hybrid solutions at a concentration of 1 mg mL$^{-1}$ in PBS and at a flow rate of 10 μL min$^{-1}$. The adsorption on the PCL surface begins immediately upon contact reaching an adsorbed hybrid molecule density of approximately 70–200 ng cm$^{-2}$ after 45 min. Such an amount corresponds to the adsorption of a full monolayer of proteins, thus indicating the formation of a dense and continuous coating on the periphery of the PCL surface. Moreover, the coating prepared from these hybrids exhibited long-term stability both in isotonic solution, PBS, and in a nutrient-rich medium such as lysogeny broth (LB) supplemented with 10% fetal bovine serum (FBS). As it is shown by the confocal images in Figure 2d, the coating was still present on the PCL fibers after 32 d of incubation in PBS and LB+FBS. Even after 180 d of incubation period, the hybrid coating did not detach from the surface as evidenced by XPS (Figure 2c and Figure S2, Supporting Information). Moreover, the coating was not damaged upon sterilization with gamma irradiation (Figure S3, Supporting Information), which further demonstrated the high stability of the coating strategy which is coupled with the strong binding of the hybrids. These results highlight the feasibility of this coating for the translation into practice.

2.1. Stealth Coating for Wound Dressings

2.1.1. Repellency to Blood Plasma Proteins

Proteins are the fastest to foul surfaces causing deleterious effects. Polymer brushes grafted from substrates have provided complete suppression of fouling. But, can the self-assembled LCI-eGFP-Polymer coatings provide similar level of protection against adsorption to render the surface stealth? We assessed the antifouling properties of these coatings by contacting them with 10% blood plasma as a mimic of the wound exudate. The latter is a complex mixture containing mainly proteins from the antifouling properties of these coatings by contacting them with 10% blood plasma as a mimic of the wound exudate. This outstanding resistance to protein fouling is comparable to the best antifouling polymer brushes in spite of relying on much thinner coatings (4–6 nm, Table S1, Supporting Information) and on a more facile application method.[12b,19]

2.1.2. Prevention of Fibroblast Adhesion

The adhesion of epithelial and endothelial cells to the wound dressing results in the conglutination of the wound bed to the wound dressing, causing pain and damage of granulation tissue every time the dressing is exchanged. For this reason, we further investigated the potential of our coating to prevent the adhesion of skin cells. In these experiments, we utilized normal human dermal fibroblasts (NHDF, 200,000 cells) which were directly seeded onto pristine PCL fiber meshes and the same fibers coated with LCI-eGFP-pCBMAA$_{912}$ as well as LCI-eGFP-pHPMA$_{343}$. Prior to cell seeding, the meshes were incubated in DMEM supplemented with 10% FBS for 3 h. Subsequently, cells were seeded on the meshes and incubated for 24 h at 37 °C and 5% CO$_2$ to give the cells the time and the optimal conditions to grow and adhere. The culture medium was enriched with 10% v/v fetal bovine serum (FBS), a natural cocktail supporting cell adhesion, making the conditions for cell repellence more challenging. After rinsing with PBS buffer to remove non-attached cells, the remaining cells were fixed with paraformaldehyde and stained for cell nuclei, f-actin (marker of the cytoskeleton) and the focal adhesion marker vinculin using immunostaining. CLSM revealed that fibroblasts could rapidly adhere onto PCL fibers in large numbers (Figure 3b). Super-resolution images acquired by stimulated emission depletion (STED) microscopy (Figure 3c) showed spindle-shaped fibroblasts with cytoplasmic extensions along the cytoskeleton (actin staining) which concentrated in focal adhesion points (vinculin staining) at the surface of the fibers clearly demonstrating the strong adhesion of the fibroblasts to the mesh. Overall, this demonstrates the promiscuity of fibroblasts for adsorbing onto surfaces previously fouled by proteins. On the other hand, only very few cells could be found throughout the meshes coated with LCI-eGFP-Polymers. Compared to the uncoated meshes, the cells found inside the coated ones exhibited a round shape and smaller size without an evident organization of the cytoskeleton (Figure 3b). Moreover, no contact points could be observed. Thus, the cells observed in the coated meshes were trapped but not attached, in stark contrast to the cells observed on the bare PCL fibers. Therefore, it is concluded that LCI-eGFP-Polymer coatings could prohibit fibroblast adhesion.

2.1.3. Prevention of Bacterial Adhesion and Colonization

One of the most dreadful outcomes for chronic wounds is infection. The objective of the dressing is to act as a barrier to the colonization, however, often bacteria can attach to the dressing itself acting as a cesspool of bacteria. Thus, we assessed the adhesion of an E. coli biofilm forming clinical isolate strain known to be one of the most common pathogens for later stages of chronic wounds capable of significant tissue damage.[20] Massive bacterial colonization, occluding almost all free space was observed for the uncoated PCL fibers (Figure 4a). This was accompanied
with the generation of extracellular polymers marking the formation of biofilm. On the other hand, hardly any bacteria could be found across the coated meshes (pHPMA792; Figure 4b). This experiment serves to benchmark the excellent repelling properties against bacterial adhesion. The antimicrobial activity of the coating is tested with \textit{S. agalactiae}, vide infra.

Furthermore, the antifouling coating could be applied on three over-the-counter commercial wound dressings, viscose and cotton gauzes, and polyurethane foam (Figure S4, Supporting Information), resulting in the prohibition of bacterial colonization even in the intricate porous structure of the foam. Thus, the application of the LCI-eGFP-Polymer to the fibers and dressing could prohibit the adhesion of these pathogenic squatters indicating the remarkable efficiency and ease of transferability of these single-molecule-thick coatings.

2.2. Kill&Repel Coating for Wound Dressings

The Kill&Repel coating was formed by the assembly of LCI-eGFP-Polymer and a specifically designed LCI-EndLys. The latter is a fusion construct of LCI with the bacteriophage endolysin PlyGBS94 (EndLys), designed to combine adhesion with high bactericidal activity. EndLys was selected due to its very high activity towards \textit{S. agalactiae}\cite{17b} a common pathogen for wounds.\cite{18a,c,e} EndLys was fused to LCI by a flexible (GGGGS)\textsubscript{3} linker to prevent negative steric hindrance that would affect the functions of each block.

We assessed the adsorption kinetics of LCI-EndLys, LCI-eGFP-pCBMAA\textsubscript{912} and LCI-eGFP-pHPMA\textsubscript{792} at the same molar concentration. The rate of adsorption was very similar during the entire course of immobilization despite the differences in molecular weight (Figure 5a, Table S2 and Figure S5, Supporting Information) and chemical composition. This indicates that the adsorption process is mediated by the binding of the same moiety, LCI, in agreement with previous reports from our group.\cite{15} Thus, the ratio of LCI-EndLys to LCI-eGFP-Polymer at the surface will be identical to the one in solution.

Furthermore, we assessed whether the co-immobilization of LCI-EndLys had an effect on the stealth properties (Figure 5b). Protein adsorption corresponding to 128 ng cm\textsuperscript{-2} was observed when the coating consisted of only LCI-EndLys, thus indicating...
the inability of LCI-EndLys to prevent protein fouling. On the other hand, only minute amounts of protein deposits (less than 28 ng cm\(^{-2}\)) were observed on the studied Kill&Repel coatings. LCI-eGFP-pCBMA912/LCI-EndLys and LCI-eGFP-pHPMA 792/LCI-EndLys coatings have reductions of 89% and 84%, respectively compared to bare PCL. These results demonstrated that the co-immobilization of LCI-EndLys did not impair the excellent antifouling properties of LCI-eGFP-Polymer. The superior resistance of the Kill&Repel coating to fouling was further evidenced in in vitro cell adhesion studies. PCL meshes coated with LCI-eGFP-pHPMA 792/LCI-EndLys successfully prevented the nonspecific adhesion of fibroblasts (Figure S10, Supporting Information).

The translation of this technology demands the inertness and the biocompatibility of the Kill&Repel coatings, which were evaluated by extraction and direct contact assays according to ISO10993. In the extraction assay, the meshes were incubated in DMEM medium for 72 h, which was then transferred to a monolayer of human skin fibroblast (70% confluent). The 24 h incubation resulted in no changes in the metabolic activity (MTS assay) of the fibroblasts indicating that no toxic components were released from the LCI-eGFP-Polymer or Kill&Repel coatings (Figure 6a). This is in agreement with stability tests (vide supra) demonstrating that the coating remained intact even after 6 months incubation in buffered saline or lysogeny broth. In the direct assay, the meshes were directly applied onto a confluent monolayer of fibroblasts and incubated for 48 h. This condition is more similar to that in practice and showed that even in close proximity, LCI-eGFP-Polymer and LCI-EndLys had no harming effect on the metabolic activity of the skin cells (no statistical difference compared to bare PCL meshes, Figure 6c). Importantly, cells continued to grow unaffected and did not show changes in morphology as evidenced by live/dead staining images (Figure 6b). This proved the great cyto compatibility of the Kill&Repel coating and underlined the safety of this novel coating strategy. But, does immobilization of the endolysin affect its lytic activity? The immobilization of enzymes on non-polar surfaces is particularly challenging, as the enzymes may denature upon adsorption, resulting in a decrease or complete loss of their activity. Therefore, we investigated the endopeptidase activity of the EndLys, i.e., the ability to hydrolyze the peptidoglycan, after it was immobilized on PCL meshes. For this test, we included meshes modified with LCI-eGFP-polymer/LCI-EndLys at a ratio of 70/30 and also LCI-EndLys in solution (3 \times 10^{-6} \text{ M} in PBS). We utilized a fluorogenic substrate, which consisted of the synthetic peptide γ-Glu-Lys-D-Ala-Ala-Ala conjugated to the (FRET) pair 2-aminobenzoyl/2,4-dinitrophenyl (Abz/Lys(Dnp)), fluorophore/quencher). The selected amino acid sequence corresponds to the cross-bridges of group B Streptococci.\(^{[21]}\) If the peptide is cleaved by LCI-EndLys, the distance between Abz and Dnp is significantly increased leading to the activation of Abz and a fluorescent signal that is used to determine the enzymatic activity (Figure 7a). A constant increase of fluorescence was observed with all modified meshes (LCI-eGFP-Polymer/LCI-EndLys), which confirmed on one hand that LCI-EndLys was co-immobilized and on the other hand that after immobilization LCI-EndLys remained active. Astonishingly, similar cleavage kinetics were observed with the LCI-EndLys in solution (3 \times 10^{-6} \text{ M}) and with the meshes functionalized with LCI-eGFP-Polymer/LCI-EndLys at a ratio of 70 to 30 mol\%. It should be noted, that the solution utilized to coat these meshes contained in total less than 2 \times 10^{-6} \text{ M} of LCI-EndLys which demonstrated that the enzymatic activity of EndLys is not dampened by immobilization, but on the contrary enhanced. Such enhanced activity is intriguing given the
Bacterial suspension without the treatment of any wound dressing, but otherwise treated under the same conditions, served as control. The Kill&Repel coating achieved a bacteria reduction of 92% after 1 h of incubation (Figure 7b). This corresponds to about 1 log reduction and indicates the efficient and rapid bactericidal activity of the Kill&Repel system. It is again remarkable that the immobilized EndLys displayed higher activity compared to the same molecularly dissolved one, where bacterial reduction amounted to 41 and 52% for concentrations of $1 \times 10^{-6}$ and $2 \times 10^{-6}$ m, confirming the same observation as for the FRET peptide, vide supra. This increase in activity may be associated with a combination of a potentiating effect caused by co-immobilization with pHMPA and multivalent effects: The immobilization of EndLys generates a very high interfacial concentration on the surface of the fiber meshes thus many EndLys can simultaneously attack the same region of an incoming bacterium thereby exerting a focused damage on the peptidoglycan and a more lethal action compared to the free enzyme.

Sessile bacteria pose an even higher challenge than their planktonic counterparts do. We simulated the infected wound by seeding *S. agalactiae* onto agar plates (OD$_{550}$ = 0.8). The functionalized meshes were applied gently on top of the agar (Figure 8a). After 24 h of incubation, the meshes were removed and the bactericidal activity was evaluated based on the presence of inhibition zones. The Kill&Repel meshes could clear the treated area eradicating 96% of bacteria (Figure 8b,c). At the same time, their surface remained uncolonized, even from residues of dead bacteria (Figure 8d). In contrast, bare PCL meshes caused only a reduction of 48% and were fully colonized by *S. agalactiae*, indicating biofilm formation (Figure 8d). The observed reduction is related to the dragging of bacterial colonies as the dressing is removed and not by an antimicrobial effect as evidenced by the fact that the fibers were fully colonized. Meshes modified with LCI-eGFP-pHPMA only, showed no further reduction in coverage than the one caused upon removal, indicating that the bactericidal activity is attributed to EndLys only (Figure 8b and Figure S9, Supporting Information). These findings demonstrated the synergistic effect of the two components of our novel coating: while LCI-EndLys can rapidly kill bacteria on contact reducing the burden of invading bacteria, LCI-eGFP-pHPMA prevents the buildup of residues of killed bacteria preventing the surface from being irreversibly blocked. Moreover, the zones of growth inhibition were confined to the contact zone (agar plate area below the dressing, Figure 8c), evidencing once again the stable adhesion of the hybrid molecules to the surface.

**3. Conclusions**

We introduced the Kill&Repel coating strategy, which synergistically combines the ability to repel pathogens with a biorthogonal bactericidal strategy that causes no harm to eukaryotic cells. The coating was formed by the molecular adsorption of LCI-eGFP-pHPMA and LCI-EndLys from diluted aqueous solution onto the substrate without the need for additional energy input. We demonstrated that the unique combination of LCI-eGFP-pHPMA with LCI-EndLys exhibited great
antifouling properties towards proteins from blood plasma, fibroblasts, and pathogenic bacteria. This performance is on par with the best polymer brushes in spite of being only few nanometers thick, while posing no cytotoxic effect to human dermal fibroblasts.

pHPMA had a boosting effect on EndLys when co-immobilized, resulting in higher enzymatic activity and improved killing of bacteria. The Kill&Repel coating reduced the concentration of planktonic bacteria by 93%, which was more efficient than the free enzyme, suggesting, that a) the close proximity of pHPMA favorably alters the structure of EndLys, increasing its enzymatic activity and b) the comparatively high density of EndLys on the surface could localize the hydrolysis of the peptidoglycan of bacteria in direct attachment to the surface, resulting in a more effective bactericidal effect than with the free enzyme. Moreover, when applied onto sessile *S. agalactiae* colonies on agar the Kill&Repel coated meshes fully cleared the surface they covered, while not allowing bacteria nor their debris to adhere on the fibers. This finding represents a significant improvement over current bactericidal strategies that kill bacteria at the expense of generating bacterial resistance, with detrimental consequences for the injured tissue.

We envision that our Kill&Repel coating system can contribute to the next generation of advanced wound dressings and provide an alternative solution to the antibiotic resistance crisis. The ease of preparation opens up possibilities for customization, e.g. PlyGBS94 can be exchanged with another endolysin that targets other microbes. Thus, it offers a universal avenue

![Figure 6](image-url)
Figure 7. a) Kinetics of the enzymatic activity of free and immobilized LCI-EndLys assessed by monitoring the increase of fluorescence intensity caused by cleavage of the FRET peptide. b) Proposed killing mechanism that endowed Kill&Repel meshes with a higher efficiency compared to free LCI-EndLys. The immobilized enzyme concentrates on the surface and kills bacteria more efficiently. c) SEM images after 1 h incubation of meshes in a concentrated S. agalactiae suspension (OD$_{550}$ = 0.8). Scale bars are 20 µm.
for imparting specific antimicrobial activity to surfaces that match application demands. Its application in the early stages of a wound could drastically reduce the risk of wound infection and development of a chronic condition.

4. Experimental Section

Materials, Cells, and Characterization: Details of materials, cells, and characterization techniques can be found in the Supporting Information. Synthesis of LCI-eGFP-Polymers: The maleimide initiator 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl 2-bromo-2-methylpropanoate was conjugated to the cysteine residue (position 69) of eGFP by Michael addition (LCI-eGFP-Initiator) as previously described.[14a] For the polymerization of LCI-eGFP-pHPMA and LCI-eGFP-pCBMAA hybrids, a 10 mL Schlenk tube was filled with HPMA or CBMAA (Table S1, Supporting Information), CuBr2 (0.05 eq.), Me6TREN (0.55 eq.) and 0.5 mL PBS. 4.5 cm of hydrazine-activated Cu wire was wrapped around a magnetic stirring bar and fixed at the neck of the Schlenk tube using a magnet. The tube was degassed by six freeze-pump-thaw circles and backfilled with Argon. The LCI-eGFP-Initiator solution (1 mL, 1 eq.) was degassed for 1 h by Argon stripping. The polymerization was started by transferring the LCI-eGFP-Initiator solution to the Schlenk tube utilizing a gas-tight Hamilton syringe. The stirring bar was dropped and the polymerization continued for 18 h at 31 °C. The polymerization was stopped by exposing the solution to air and the addition of 0.5 mL of PBS. The reaction mixture was purified with Amicon Ultra Centrifugal Filters (10 kDa). The conversion and purity of the hybrids were determined via 1H-NMR spectroscopy (Table S1 and Figure S1, Supporting Information). Production and Purification of LCI-EndLys: 3xGGGGS-LCI was amplified by Polymerase Chain Reaction and subsequently fused to the C-terminus of StrepII-PlyGBS94 using the NEBuilder HiFi DNA Assembly Kit (New England Biolabs). For overexpression, the plasmid was then transformed into chemically competent E. coli BL21 (DE3) Gold cells and successful fusion of LCI and endolysin was confirmed by Sanger sequencing.
(Eurofins Genomics). 5 mL LB were inoculated with E. coli BL21 (DE3). Gold cells at 37 °C for 16 h. The working culture (400 mL) was prepared by diluting the inoculum in terrific broth to an optical density (OD600) of 0.05 and cultivated for an additional 3 h at 37 °C with aeration at 180 RPM until reaching OD600 of 0.6. Protein over-expression was induced by supplementing isopropyl β-D-1-thiogalactopyranoside (0.1 x 10^-3 m final concentration) at 20 °C. After 20 h cells were harvested by centrifugation at 11 200 g for 30 min and 4 °C. Cell pellets were suspended in Strep-tag II wash buffer and disrupted by sonication on ice (3 min, interval 10 s, 70% amplitude). Soluble proteins were collected after centrifugation at 3200 g for 45 min (4 °C). The protein solution was filtered through a 0.45 µm cellulose-acetate filter (Cytiva) and purified using gravity-flow chromatography with a prepacked affinity chromatography column (StrepTrap HP, 5 mL, Cytiva). Samples were dialyzed two times against PBS (pH 7.4) using a dialysis tube with a pore size of 3.5 kDa. Protein concentration was determined with the BCA protein assay kit (Novagen, Merck KGAA) and protein homogeneity was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a Tris-Glycine gel (4–20%; NuSep Inc.)

**Formation of Coating on PCL Meshes:** The coating was formed by immersing the meshes (thrice washed with PBS, 5 min) into LCI-eGFP-Polymer solution (46 µmol L^-1 in PBS). After 60 min of incubation at 4 °C, the meshes were rinsed with copious amounts of PBS.

The formation of the Kill&Repel coatings was formed as above but replacing a fraction of the LCI-eGFP-Polymer with LCI-EndLys to achieve the ratio 90/10 and 70/30 mol/mol.

**Adhesion Assay of E. coli:** A single colony of E. coli DSM-1103 was inoculated in 10 mL Mueller Hinton Broth and grown for 12 h (37 °C, 180 RPM). For the adhesion assay, the MHB was supplemented with 10% PBS and the optical density was adjusted to OD600 of 0.1, corresponding to approximately 10^8 CFU mL^-1. The bare and functionalized meshes (1 cm x 1 cm) were placed in 15 mL Falcon tubes and covered with 3 mL of bacterial suspension (OD600 = 0.1). All types of meshes were tested in triplicates. The tubes were closed and placed inside the incubator for 24 h at 37 °C and 180 RPM. Planktonic cells were removed by pipetting out the cell suspension. The meshes were washed four times with 2 mL of PBS (5 min) and fixed in 3% glutaraldehyde in PBS (overnight, 4 °C). All samples were dehydrated through a graded ethanol series (25%, 50%, 70%, 90%, and 100% dry, 15 min each step, 2 x 100%) and air dried using hexamethyldisilazane. Adhesion of E. coli was visualized by SEM.

**Adhesion Assay of NHDF:** Cells were cultured in DMEM supplemented with 10% FBS until passage 6 and harvested by trypsin treatment. Prior to cell seeding, bare and modified meshes were incubated overnight in PBS and for 3 h in complete cell medium at 37 °C. Meshes were placed in 24-well plates and 200,000 fibroblasts were seeded onto each substrate (300 µL DMEM). Samples were incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO2. Subsequently, cells were washed, fixed, and permeabilized for staining of cellular components. For evaluation of cell morphology and structure, the cell nucleus, actin filaments, and vinculin were stained as described in the Supporting Information. Microscopic evaluation was carried out using a Leica TCS SP8 confocal microscope (Wetzlar, Germany).

**Bactericidal Activity against Planktonic S. agalactiae:** 20 mL THB was inoculated with one single colony of S. agalactiae DSM-2134 and incubated statically for 17 h at 30 °C. A 1:100 dilution was statically grown (37 °C) until reaching an optical density OD550 of 0.4. The experiment was conducted in a 50 x 10^-3 m ammonium acetate buffer containing 10 x 10^-3 m calcium chloride and 1 x 10^-3 m DTT (lysine buffer, pH 6.2). The cells were washed by centrifugation (2 x PBS, 1 x lysin buffer, 10 min each time) and resuspended in lysin buffer to an optical density OD550 of 0.8. For testing the free endolysin, 2 mL Eppendorf tubes were filled with 400 µL cell suspension (OD550 = 0.8) and 500 µL LCI-lysinBS94 at concentrations of 1 x 10^-6 and 2 x 10^-6 in lysin buffer or 500 µL lysin buffer as control. The Kill&Repel meshes were tested by placing a mesh (3 cm x 2 cm) inside a 2 mL Eppendorf tube and covering it with 750 µL cell suspension (OD550 = 0.8) and 750 µL lysin buffer. Samples were incubated for 1 h at 37 °C on a 3D shaker. 900 µL of each sample were taken and the reduction of optical density OD600 was measured. Mes...
