Lectin-Functionalized Composite Hydrogels for “Capture-and-Killing” of Carbapenem-Resistant *Pseudomonas aeruginosa*

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**ABSTRACT:** Infections with multiresistant pathogens are a leading cause for mortality worldwide. Just recently, the World Health Organization (WHO) increased the threat rating for multiresistant *Pseudomonas aeruginosa* to the highest possible level. With this background, it is crucial to develop novel materials and procedures in the fight against multiresistant pathogens. In this study, we present a novel antimicrobial material, which could find applications as a wound dressing or antimicrobial coating. Lectins are multivalent sugar-binding proteins, which can be found in a variety of plants and bacteria, where they are associated with biofilm formation. By immobilizing lectin B on a protein-based hydrogel surface, we provided the hydrogel with the ability to immobilize (“catch”) pathogens upon contact. Furthermore, another hydrogel layer was added which inhibits biofilm formation and releases a highly potent antimicrobial peptide to eradicate microorganisms (“kill”). The composite hydrogel showed a high antimicrobial activity against the reference strain *Pseudomonas aeruginosa* PAO1 as well as against a carbapenem-resistant clinical isolate (multiresistant Gram-negative class 4) and may thus represent a novel material to develop a new type of antimicrobial wound dressings to prevent infections with this problematic pathogen of burn or other large wounds.

**INTRODUCTION**

Antimicrobial infections have become one of the major health issues of our century, especially with nosocomial infections being among the most challenging tasks to handle for healthcare facilities. While plenty of literature deals with this problem and hundreds of research facilities worldwide are working on this topic, no country or institution can claim to be able to solve this problem or to come up with an ultimate solution in a reasonable time for the future. According to the World Health Organization (WHO), infections are the second highest cause for mortality worldwide, and according to the European Center for Disease Control (ECDC), more than 4 million patients are affected by nosocomial infection every year in Europe alone, § with immune-suppressed patients especially being life-threatened. In early 2017, carbapenem-resistant *P. aeruginosa* strains were classified to be among the most threatening pathogens with the highest demand for novel antibiotics by the WHO. ‡ Due to these alarming numbers and facts, novel procedures, methods, and materials must be developed to keep this tremendous health problem at bay.

Hydrogels, 3D networks which can incorporate huge amounts of water while being insoluble, have recently attracted increasing attention as a unique class of biomaterials. They are soft materials which have recently been used frequently in cosmetics, ‡ for drug delivery, ‡‡‡ and for tissue engineering. ‡‡‡ Most hydrogels have flexible properties and can easily be casted into the desired form. Additionally, many hydrogel systems can be modified and designed according to the intended use by adjusting their physio-chemical properties and by functionalizing the material postproduction to add antimicrobial features. In this context, hydrogels with inherent antimicrobial activity, e.g., positively charged polymers, ‡‡‡‡‡ are distinguished from hydrogels with acquired antimicrobial activity, e.g., by encapsulating with or coupling to antimicrobial compounds, like antibiotics, antimicrobial peptides (AMP), or nanoparticles. ‡‡‡‡‡‡ These unique material features qualify hydrogels as promising and potent materials in the fight against pathogens at many fronts, and several studies have been presented on the use of hydrogels as depots for drug delivery ‡‡‡‡‡‡‡ or as...
antimicrobial coatings. Furthermore, there are numerous examples of hydrogels as biosensors, where the surface of a hydrogel is functionalized to enable bacterial binding and detection. This immobilization of microorganisms can be realized with different mechanisms, e.g., with aptamers, antibodies, concanavalin A, or porous surfaces, which can capture bacteria. Generally, the main purpose is the immobilization of bacteria to enable detection and classification techniques for pathogens with the aim to reduce detection limits to optimize sensitivity.

In this study, however, a “capture-and-kill” approach was intended, developing a concept in which high numbers of pathogenic bacteria can be immobilized on a hydrogel surface, followed by subsequent killing of the attached organisms. A composite hydrogel material was used, which combines and exhibits different antimicrobial features. A proximal (upper) hydrogel layer facing the infection site is responsible for the bacterial immobilization, while a distal (lower) layer exhibits antimicrobial effects, resulting in subsequent killing of pathogens. This material could serve as a novel compound in composite wound dressings. While exudate is absorbed from the wound, pathogens are immobilized onto the hydrogel and subsequently killed by the (released) compound. Especially for severe infections with multiresistant bacteria, this would significantly facilitate the burden and thus possibly enable or, at least, support the immune system to overcome the infection on its own.

For the proximal layer in our concept, a serum albumin-based protein hydrogel was used. Serum albumin is among the most abundant proteins in the human body and is responsible for the maintenance of the oncotic pressure. The formation of the hydrogel matrix is assisted by the four-armed amine reactive cross-linking agent, tetrakis (hydroxymethyl) phosphonium chloride (THPC). Within minutes, water insoluble, highly resilient hydrogels can be formed from different protein backbones. These biocompatible hydrogels are easy to handle due to their high mechanical stability, easy to produce due to the straightforward use of a liquid two component system to initiate gel-to-sol transition, can be casted into any possible form, and exhibit high stabilities toward external stimuli (pH, temperature, etc.). Furthermore, the material can easily be modified postproduction due to the numerous reactive hydroxy-, amine-, or thiol-groups in the BSA molecules, which can serve as targets for the introduction of novel, potentially antimicrobial, moieties into the hydrogel. This highly resilient layer is equipped with the capability of catching pathogenic microorganisms. For this purpose, the hydrogel was modified with lectin molecules, a class of sugar binding proteins, which are present in many plants and bacteria, and are capable of binding several sugar moieties at once with varying affinities for different types of sugars due to their multivalency. In bacteria, lectins are associated with cell recognition processes, especially in biofilm formation. As they are capable of binding to a variety of glycosylated structures as well as sugar moieties of the bacterial surface, they can be used in the detection and immobilization of bacteria. In our setup, a fusion protein of the P. aeruginosa tetrameric lectin B (LecB) and YFP as a reporter domain, which has recently been published as an adaptor for the reversible immobilization of human cells, was in the application presented here, covalently bound to the surface of the hydrogel backbone via thiol reactive cross-linker molecules, while maintaining its functionality concerning fluorescence and sugar binding capability. By using this approach, which was inspired by the lectin-mediated cell recognition and binding processes in the biofilm formation of several pathogens, bacteria could be immobilized on the proximal hydrogel layer via sugar-mediated binding to the immobilized lectin.

The second and distal part of the composite material is composed of a fibril-forming amino acid hydrogel. Fmoc-protected phenylalanine (fmocF) has been described to form fibrils under physiological conditions, which can bind water at very low concentrations (0.1% (w/v)) with one molecule being capable of entraping 21500 water molecules, making it a highly potent supergelator. Compared to the proximal protein hydrogel layer, these fibrillar hydrogel materials can bind 25–200-fold more water based on the dry substance, which qualifies them as very potent water reservoirs, which are typically essentially required to build up wound dressing layers. The prototypic example, fmocF, is obtainable and affordable in the kilogram range and readily forms hydrogels under physiological pH values and temperatures due to π–π interactions of the ring structures. Due to this very gentle gelation procedure, it is a convenient material for the encapsulation of a variety of antimicrobial agents. Furthermore, the enantiomer of L-phenylalanine has been shown to prevent or disassemble biofilm of several pathogenic microorganisms, e.g., Staphylococcus aureus and P. aeruginosa, while maintaining its potential to form hydrogels. To further increase the antimicrobial effect of the composite hydrogels, the BP100-based AMP C14R was incorporated into the lower layer of the hydrogel. BP100 was originally derived from a library of cecropin–melittin (apo toxin derived from bee venom) peptides. Recently, Torcato et al. reported on a novel BP100 analogue with a significantly improved activity against both Gram-negative (E. coli, Klebsiella pneumoniae, and P. aeruginosa) and Gram-positive (S. aureus, Streptococcus pneumoniae, and Enterococcus faecium) bacteria due to its interaction with and disruption of the bacterial membrane.

Confocal laser scanning microscopy (CLSM) revealed a stable immobilization of the lectin onto the material, while a subsequent catching of P. aeruginosa PAO1 and E. coli Tuner (DE3) was performed with an optimized lectin concentration of 250 μM. Fmoc-D-F showed to have inhibitory effects on the biofilm formation of P. aeruginosa PAO1, while encapsulated AMP showed to have strong antimicrobial effects on both E. coli Tuner (DE3) and P. aeruginosa PAO1. The composite material could be used to catch and kill both E. coli Tuner (DE3) and P. aeruginosa PAO1 cells. To evaluate the potential of this concept for the treatment of infections with multidrug-resistant bacteria, we also evaluated a clinical Pseudomonas aeruginosa isolate. This strain expressed the New Delhi metalloproteinase-type carbapenemase and was classified as “4MRGN” with resistance against all four classes of antibiotics (acylaminopenicillines, cephalosporines, carbapenems, and chinolones), and it is available for first line therapy against Pseudomonas. All of the results indicate a successful immobilization and subsequent killing of the relevant strain for both the well-described laboratory strains as well as for freshly isolated, carbapenem-resistant P. aeruginosa PAO1, which has recently been classified as a critical risk factor for human health with the highest priority for the development of novel antimicrobial strategies.

**MATERIALS AND METHODS**

Ampicillin, tetracycline, bromophenol blue, CaCl₂, K₂HPO₄, KCl, KOH, KH₂PO₄, LB medium, lysozyme, HCl, NaCl, and tris were ordered from Roth (Carl Roth GmbH and Co. KG, Karlsruhe,
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with PBS, pH 7.4. For mixed hydrogels, fmoc-D-F–OH and fmoc-L-FF–OH were mixed 1:1 (w/w) and diluted in DMSO prior to the hydrogel preparation. Tubes were stored at 4, 21, and 37 °C. At the appropriate times, hydrogel was removed from the solution, dried with a highly absorbent paper, and weighed.

**Catching of P. aeruginosa and E. coli on Hydrogel.** *P. aeruginosa* and *E. coli* were grown to an OD<sub>600</sub> of 0.1 in LB supplemented with 100 μg/mL of ampicillin, induced with 1 mM isopropyl-β-d-thiogalactoside (IPTG), and transferred onto a BSA hydrogel functionalized with different concentrations of LecB (see above). The hydrogel was incubated with 30 μL of bacterial solution (OD 1) for 30 min, followed by being washed twice with PBS to remove unbound bacteria. The potential to capture bacteria with different LecB concentrations was analyzed with a Zeiss Confocal Microscope (Carl Zeiss AG, Oberkochen, Germany) at 405 nm for *P. aeruginosa* and 488 nm for *E. coli*.

**Toxicity of the Drug-Loaded fmoc-D-F–OH Hydrogel on P. aeruginosa and *E. coli***. To test the toxicity of the hydrogel, fmoc-D-F–OH hydrogel alone and fmoc-D-F–OH hydrogel loaded with AMP were prepared as described above. An overnight culture of *P. aeruginosa* PAO1<sup>55,56</sup> and *E. coli* Tuner (DE3) was diluted to 1<sup>10</sup> CFU/ml and 200 μL of the cell suspension was incubated with 100 μL of the drug-loaded hydrogel for 4, 8, 16, and 24 h. As a control, cells were spiked with the adequate amount of PBS instead of a hydrogel. After incubation at 30 °C and 200 rpm, 100 μL of the samples was transferred into a 96-well plate, spiked with 20 μL of 0.1% resazurin, covered with aluminum foil, incubated for 2 h, and measured with a Tecan200 M fluorescence reader (Tecan Group Ltd., Männedorf, Switzerland) at an excitation wavelength of 535 nm and an emission wavelength of 595 nm.

**Composite Hydrogel Effect on Multi/Carbapenem-Resistant *P. aeruginosa***. Composite hydrogels loaded with AMP and functionalized with YFP-LecB were produced as described above, as well as composite hydrogels without YFP-LecB and composite hydrogels without AMP. Carbapenem-resistant *P. aeruginosa* (10<sup>6</sup> cells/mL, 100 μL) cells were transferred onto the hydrogel. After 2 h, the bacteria were washed subsequently with 200 μL of PBS, followed by being washed with 200 μL of PBS containing 20 mM mannose. Bacteria were plated on LB agar plates and grown overnight at 37 °C. The next day, the CFUs were counted with FIJI software.

**Statistical Analysis.** All error bars represent standard deviations. Statistical significance was tested with a student’s t test with α = 0.5.

### RESULTS AND DISCUSSION

LecB is a tetrameric protein which is known to bind different sugars with different affinities and is used by the producing bacteria to immobilize themselves on abiotic surfaces, for example, during biofilm formation or the establishment of infections.<sup>37,47</sup> This natural concept has been used to develop

Figure 1. Schematic illustration of the capture-and-kill mechanism of the hydrogel material. The top hydrogel layer consists of bovine serum albumin (BSA) chemically cross-linked with THPC and is subsequently surface functionalized with a recombinant fusion protein of *P. aeruginosa* PAO1 LecB and YFP as a reporter domain to enable the reversible immobilization of pathogenic bacteria on its surface. The bottom layer consists of fmoc-protected ε-phenylalanine hydrogels, which are loaded with an AMP. In a two-step reaction, bacteria are first immobilized on the hydrogel surface, followed by subsequent sequestration of the AMP from the lower hydrogel and a consequent killing of the immobilized microorganism.

Figure 2. Hydrogel modification and catching of bacteria. (A) The top line shows an unfunctionalized piece of hydrogel, while the lower layer shows a piece of the functionalized material. The hydrogel is functionalized with a 10 μM bispecific maleimide cross-linker, which targets cysteines in the yellow fluorescent protein–LecB (YFP-LecB) fusion product for 16 h. YFP-LecB (62.5–500 nM) is immobilized for 2 h on the hydrogel surface while maintaining its functionality and visualized with a Zeiss laser scanning confocal microscope at 514 nm. (B,C) CLSM of *E. coli* Tuner (DE3), which intracellularly expresses eGFP, was visualized at 405 nm (B), and *P. aeruginosa*, which intracellularly expresses CFP, was visualized at 405 nm (C). The modified hydrogel was incubated with bacteria for 2 h, followed by extensive washing with PBS.
lectins for the immobilization of bacteria on surfaces to produce sensors for the detection of bacteria. We decided to directly use the lectin-mediated immobilization of bacteria for the construction of a composite material for the catching of human pathogens on synthetic surfaces, like a hydrogel modified with LecB from P. aeruginosa. The combination of lectins and composite hydrogels may offer the possibility to develop novel antimicrobial wound dressings, especially for the treatment of early infected wounds. The principal behind this would be an immobilization of the pathogens on the wound surface, thereby resulting in their local reduction and decreasing the infective threat for the affected tissue. In a second step, the immobilized pathogen, in our concept, would be eradicated by a suitable drug, for example an AMP (Figure 1).

The modification of the hydrogel with YFP-LecB was performed with a dimaleimide linker which readily reacts with cysteines under physiological conditions. To investigate the successful modification with LecB, a variant of the protein was produced, representing a translational fusion construct with an additional yellow fluorescent protein domain (YFP) at the N-terminus of LecB (YFP-LecB). This YFP contains two naturally occurring cysteine residues in its 3D structure, one of which is located in the center of the barrel structure, while the other is solvent accessible on the outer surface (PDB: 2JAD) and is thus available for further cross-linking reactions, e.g., with a maleimide linker reagent. The modification of the material surface was analyzed by measuring the fluorescence of the YFP domain of the fusion construct using CLSM (Figure 2A). This modification appears to be considerably stable and robust since it is maintained even after extensive washing steps. Because the lectin can bind to a variety of different sugars in bacterial surface structures, it was reasonable to expect that direct capturing of bacterial pathogens could be probable on a surface which is coated with functional sugar-binding moieties. Different LecB concentrations were used to determine the immobilization of bacteria in combination with E. coli and P. aeruginosa, two well characterized bacterial strains, which were labeled by the expression of eGFP (E. coli) and CFP (P. aeruginosa). For lectin concentrations below 62.5 nM, a significant difference in the potential of the gel to catch bacteria could not be observed. However, with increasing concentrations, more E. coli and P. aeruginosa were detected on the hydrogel surface (Figure 2B,C). The best results were achieved for 250 nM, indicated by a situation where both P. aeruginosa and E. coli cells densely covered most of the surface of the materials. As for the binding capacity, approximately $4 \times 10^7$ bacteria could be immobilized per square mm hydrogel (quantified using the FIJI software, version 1.51d). Above that, higher concentrations did not increase binding capacities but unexpectedly an overall decrease of the organisms was detected, accompanied by the formation of large gaps with no measurable bacterial fluorescence on the hydrogels surface. This "peeling" may most probably be...
explained by the formation of microcolony-like bacterial aggregates mediated by the natural occurring onset of biofilm formation (probably mediated also by LecB), which can be suspected to be exposed to significantly higher shear forces during washing and is thus detached from the surface.

Since several pathogens can form stable biofilms on biotic and abiotic surfaces, depending on their initial cell titer and the incubation time with these biofilm architectures serving to protect them from external environmental factors, this would inevitably lead to the undesired growth of an even more potent reservoir of pathogens. Therefore, we added an additional functionality to the functionalized BSA hydrogel, represented by a second distal layer. This second layer is composed of fmoc-protected d-phenylalanine (fmoc-D-F) or its dipeptide fmoc-protected d-diphenylalanine (fmoc-D-FF), which both readily form hydrogels under physiological conditions by π–π interactions between the aromatic rings44 and exhibit slight antimicrobial effects.45 As the most basic but important material property, the stability of the resulting hydrogels manufactured from fmoc-D-F, fmoc-D-FF was measured as the residual wet weight after incubation in solution as described earlier.30,31 The physical principal behind the formation of hydrogels by fmoc-D-F or other protected small aromatic amino acids is the formation of nanofibrils between the monomers by π–π stacking.45 Hydrogels formed from fmoc-D-F were significantly less stable compared to their counterparts composed of pure fmoc dipeptide (fmoc-L-FF) (Figure 3A). However, it was possible to increase the stability of fmoc-D-F hydrogels significantly by the addition of fmoc-L-FF, which may be an effect of the additional aromatic ring possibly serving as a molecular branch connection to increase the degree of cross-linking between individual nanofibrils, which in turn may result in more stable materials. However, the exact mechanism has yet to be clarified.35,47 For the intended application of the hydrogel system as an antimicrobial wound dressing, the lifetime of a few days, as observed in our experiments, may already be in a sufficient range, as changing wound dressings is typically required after several hours or a few days. Another beneficial feature of using the non-natural d-enantiomer of phenylalanine is its antimicrobial effect.48,49,66 As Figure 3B indicates, the fmoc-protected enantiomers of phenylalanine already severely influence the viability of P. aeruginosa, as well as of E. coli, as model organisms. Additionally, fmoc-D-F has been described as a potent inhibitor of biofilm formation and is known to promote biofilm disruption.48–50 As biofilms might act as a protective barrier which can shield bacteria from harmful agents, such as antibiotics or peptides,67,68 it would be beneficial to prematurely avoid spoiling and subsequent undesired biofilm formation of the material.

Figure 3C,D shows the comparison of normal biofilm formation of P. aeruginosa, one of the most potent biofilm producers,69,70 and biofilm formation in the presence of fmoc-D-F. Crystal violet stains bacteria in a deep blue color71 and visualizes the biofilm formation of P. aeruginosa (Figure 3D),

**Figure 4.** Effect of encapsulated AMP. (A) To test the effect and optimal concentration for the used strains with the AMP, P. aeruginosa PAO1 and E. coli Tuner (DE3) were incubated with different concentrations (0–8 μg/mL) of AMP overnight, followed by the determination of the cell viability 0.1% resazurin at an excitation wavelength of 535 nm and an emission wavelength of 595 nm. (B) Time and concentration dependent effects of AMP encapsulated into fmoc-D-F hydrogels. After 24 h of incubation with 8 μg/mL, no more cell viability could be detected for both P. aeruginosa PAO1 (B) and E. coli Tuner (DE3) (C).
A short, cationic AMP derived from the peptide BP100 (MIC $7.5\mu g/mL$) is a fast-acting bactericidal compound in order to kill bacteria and materials surface, it is necessary to involve an effective eGFP-labeled $P.$ aeruginosa.

Cells were further stained with 0.1% resazurin, resorufin was excited at 554 nm, and viable cells are indicated in red. For $E.$ coli Tuner (DE3), no more viable cells were observed for $4\mu g/mL$ of the peptide concentration and higher, while for $P.$ aeruginosa PAO1, no more viable cells were detected for $8\mu g/mL$ of peptide concentration.

To equip the composite hydrogel system with this strong antimicrobial effect, the AMP was encapsulated into fmoc-D-F hydrogels, which could easily be polymerized under physiological conditions in the presence of the peptide. Subsequently, the release of the peptide from the material and the time-dependency of the antimicrobial effect were investigated by incubating the pathogens in the presence of peptide-loaded hydrogels harboring the same peptide concentrations (0−8 μg/mL) as used in the mentioned setup in liquids (Figure 4A).

Samples were taken after 4, 8, 16, and 24 h from bacterial solution, and the viability of the pathogens was analyzed. The concentrations, which were found to eradicate all bacteria in liquid culture, proved to be efficient also for the drug-loaded hydrogels, indicated by the fact that the killing of $E.$ coli or $P.$ aeruginosa approximated 100% after 16 h (Figure 4B,C).

After testing the proximal (catching) and distal (killing) layers alone, the complete concept with both layers unified in a functional composite material was evaluated. The hydrogel was produced by first polymerizing the distal fmoc-D-F layer, followed by polymerization of the proximal second, protein hydrogel layer directly on top of it, resulting in a mechanically stable sandwich. $P.$ aeruginosa and $E.$ coli, intracellularly expressing eGFP and CFP, were incubated with the hydrogel system, and their viability regarding different peptide concentrations was investigated using CLSM. To additionally monitor the viability of the immobilized bacteria, resazurin was used to stain the microorganisms. Resazurin is a cell permeable dye which is reduced by dehydrogenases in living organisms to resorufin, which emits red light upon excitation. Therefore, living organisms emit red light as visible in Figure 5A for $E.$ coli and Figure 5B for $P.$ aeruginosa, while dead cells do not show any red fluorescence. With increasing peptide concentrations, the viability decreased drastically until the complete disappearance of the visible living pathogens from the hydrogels surface, indicating the successful release of the AMP and the subsequent killing of the immobilized bacteria with a high efficiency.

To evaluate the potential of this concept for the treatment of infections with highly resistant bacteria, we next evaluated a clinical isolate from the lesion of the left foot of a 61-year-old patient who was bitten by a scorpion during a holiday in Kenya. The strain expressed the New Delhi metalloproteinase-type carbapenemase and was resistant to all of the tested antibiotics, patient who was bitten by a scorpion during a holiday in Kenya. The strain expressed the New Delhi metalloproteinase-type carbapenemase and was resistant to all of the tested antibiotics, with the exception of colistin. Despite intensive systemic and local treatment, this exceptionally resistant and aggressive $Pseudomonas$ strain caused extensive necrosis, and the affected lower limb had to be amputated to prevent life threatening septicemia. The 4MRGN $P.$ aeruginosa, which was isolated from a patient, was incubated for 2 h with the composite hydrogel,

![Figure 5. Effect of the drug-loaded hydrogel on pathogens.](image)
followed by sequential and extensive washing of the hydrogel surface with PBS and a mannose-containing solution. Mannose is a sugar with a high affinity for LecB and can thus replace the glycosylated structures on the bacterial surfaces, resulting in the elution of the bacteria from the hydrogel surface.

To get a complete view, three different variants of materials were used. The first one was neither functionalized with LecB nor loaded with AMP and thus served as a negative control. Pathogens were not immobilized on the surface and could be completely removed with PBS (Figure 6). Subsequently, eluted cells were plated on LB agar plates to evaluate their viability in a CFU assay (Figure 6). A subsequent additional washing step including mannose, however, did not increase the number of removed bacteria and CFU in the plate assay. In a second experiment, the material was modified with LecB but not loaded with AMP, resulting in a fully viable bacteria on the surface. Only marginal amounts of cells could be removed by washing with PBS, whereas when mannose was included in the washing solution, bacteria were eluted almost completely (Figure 6). In the third experiment, hydrogels both functionalized with LecB and loaded with AMP were used. Again, bacteria could not be removed with PBS, while bacteria, which were removed with a mannose-containing solution, did not show any growth on the plates, leading to the conclusion that the used AMP already killed all of the bacteria on the hydrogels surface (Figure 6).

In summary, the novel composite hydrogel does not only work like shown in our proof-of-principle experiment involving the common, well-characterized but honestly less harmful laboratory strain \( \text{P. aeruginosa} \ PAO1 \) but also for clinically relevant, multiresistant bacteria of the genus \( \text{P. aeruginosa} \). As the capturing step of the cells is mediated by the LecB-dependent binding of sugar residues in the polymeric structures on the bacterial cell surface and LecB has a relatively broad specificity regarding its (sugar) target, it can be expected that a variety of bacteria can be bound by LecB. Furthermore, at present, there is a huge variety of lectins,\(^7^3\) which may be of certain interest for capturing specific bacteria. Since the capturing layer of our system can easily be functionalized with probably any protein via amine- or thiol-reactive cross-linking agents, as described here, it is principally open to be adapted to every cellular target,

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Figure 6. Effect of the composite hydrogel on MRGN4 \( \text{P. aeruginosa} \). Testing of the material with multiresistant Gram-negative class 4 \( \text{P. aeruginosa} \). Three different hydrogel variants ((1) no LecB; (2) no AMP, only LecB; and (3) both AMP and LecB) proved the feasibility of the designed material and the usage against the multiresistant pathogens.
depending on the adapter protein used, with lectins being only one example. We consider the novel capture-and-kill material presented here to be a promising tool in the fight against the increasing threat from multiresistant pathogens, which may serve as a starting point to develop tailor-made wound dressings to avoid or treat clinical infections with these pathogens in the future.

## CONCLUSION

A composite material was developed which combines a protein hydrogel functionalized with LecB and a fibrillar fmoc phenylalanine-based gel as a reservoir for water and antibacterial compounds for the later development of novel wound dressings. The functionality of the lectin-mediated binding of *Pseudomonas aeruginosa* laboratory strains and clinical isolates to the material was demonstrated. The killing of the caught bacterial cells by the fibrillar and drug-loaded compartment was accomplished and shown in different experiments. We believe that this catch-and-kill concept will help to overcome problems in the surgical care of larger wounds in hospitals with these aggressive, carbapenem-resistant strains, since a dangerous and essential mechanism for the establishment of severe infections is inhibited because the formation of elaborate biofilms is avoided.

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**Notes**
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