Functional Surfaces

DOI: 10.1002/anie.200801570

Antimicrobial Surfaces through Natural Product Hybrids**

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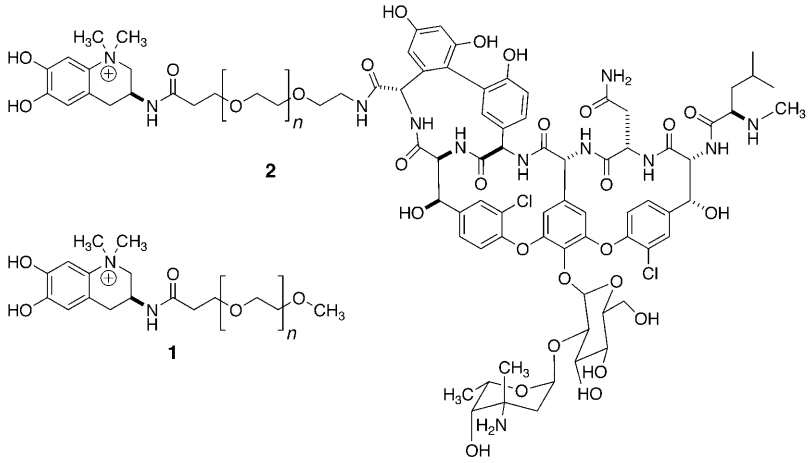
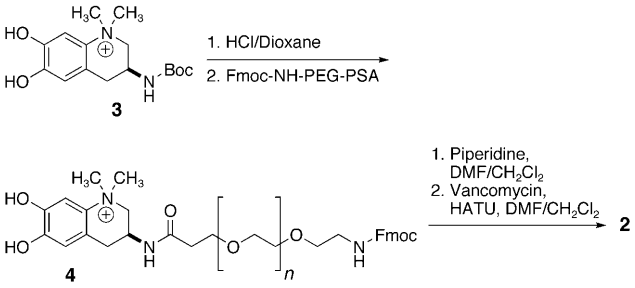
Infections after treatment in hospitals and nursing homes (nosocomial infections) pose a significant threat to patients,[1] a problem that is further accentuated by the increase in resistant pathogens.[2] High infection rates are associated in particular with implants, catheters, and stents;[3] for example, the infection rate for urinary catheters has increased up to 30% per week.[4] The encapsulation of implants by surrounding tissue adds further complications: Antibiotics have difficulty in reaching their site of action, which leads to an up to 1000-fold decrease in their efficiency.[2a,d,e,5] As a direct consequence, the replacement of implants, which implicates large costs and suffering for patients and their families, often remains the therapy of choice. An attractive approach for the prevention of such nosocomial infections lies in the attachment of antibiotics to biomaterials.[6] Herein we report the design, preparation, and biological evaluation of a natural product hybrid for the generation of antimicrobial surfaces.[7]



Natural product hybrids are compounds in which biologically active fragments of two different natural products are combined with the goal of merging different modes of action synergistically.[8] For example, cytotoxic derivatives of CC-1065 were hybridized with DNA-binding natural products[9a,b] or carbohydrates[9c] to provide highly selective potent compounds. We reported recently that derivatives of the cyanobacterial siderophore anachelin[10] are able to bind strongly to metal-oxide surfaces.[11] Thus, protein-resistant surfaces of TiO_2 were prepared by dip-and-rinse procedures in solutions of the poly(ethylene glycol)-substituted anachelin chromophore **1**.[11]

We wondered whether anachelin, with its strong surface-binding properties, could be hybridized with an antibiotic natural product. As a target molecule, we chose the hybrid **2**, in which the anachelin chromophore is linked through a poly(ethylene glycol) (PEG) linker to the clinically used antibiotic vancomycin. Each of these fragments should contribute a desired function to hybrid **2**: The anachelin chromophore should enable the immobilization of the hybrid on the surface; vancomycin interferes with cell-wall biosynthesis and should thus inhibit the growth of bacteria; and the long PEG-3000 linker should make the modified surfaces resistent to proteins and cells and ensure the optimal positioning of the antibiotic on the surface.

The synthesis of target compound **2** commenced with the preparation of the anachelin chromophore **3** from Boc-L-DOPA by a known procedure (Scheme 1).[10c,d,g] Removal of the Boc group (with HCl in dioxane) and subsequent coupling to the bifunctional Fmoc-NH-PEG-succinidyl ester resulted in the PEG-substituted anachelin derivative **4**. Cleavage of the Fmoc group under mild conditions with piperidine gave the terminal amine, which was coupled to vancomycin according to a modified literature procedure by using the reagent HATU.[12] The resulting hybrid **2** was purified by size-exclusion chromatography.



Scheme 1. Preparation of the hybrid **2**. DMF = *N*,*N*-dimethylformamide, Fmoc = 9-fluorenylmethoxycarbonyl, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; PSA = propionic acid *N*-hydroxysuccinidylimide anhydride; Boc = *tert*-butoxycarbonyl, L-DOPA = L-3,4-dihydroxyphenylalanine.

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[**] We thank the Swiss National Science Foundation for support (project no. 200021-115918/1). This work is part of the planned dissertation of S.B. at ETH Zürich. We thank B. Malisova for the preparation of the Ti chips.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200801570.

We first investigated whether the modification of vancomycin to form the hybrid **2** had an impact on its biological activity. Both vancomycin and **2** were tested in disk-diffusion experiments on *Bacillus subtilis* ATCC 6633. The hybrid **2** retained the antimicrobial activity of the parent compound, albeit decreased by roughly a factor of two. This decrease can be explained by the higher molecular weight and the resulting lower diffusion rate of **2**.

We then investigated the surface modification of TiO_2 with the hybrid **2**. Titanium is frequently utilized in implants owing to its favorable properties as a biocompatible material. We functionalized TiO_2 surfaces by using an operationally simple procedure developed previously for the anachelin chromophore.[11] The TiO_2 chips were incubated in a solution of **2** in MOPS (3-(*N*-morpholino)propanesulfonic acid) buffer for 4 h and then washed with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer. This dip-and-rinse procedure enabled not only the successful functionalization of TiO_2, but also that of glass slides.

The resulting functionalized TiO_2 surfaces were evaluated for their biological activity. We used the live/dead kit,[13] which enables the differentiation of live and dead cells by fluorescence microscopy. Whereas living cells are stained green, the DNA in dead cells is labeled with a red fluorescent dye. As a model organism, we chose *Bacillus subtilis* ATCC 6633,[14] which is susceptible to vancomycin.[15] Noncoated TiO_2 chips incubated in bacterial suspensions served as a control. Viable cells were detected when these chips were stained with the live/dead kit (Figure 1 a). As a positive control, an untreated TiO_2 surface was incubated with *B. subtilis* in the presence of dissolved vancomycin; only dead cells were detected by fluorescence microscopy after staining (Figure 1 b).[16]

After establishing these positive and negative controls, we incubated TiO_2 surfaces functionalized with **2** in suspensions of *B. subtilis*. After incubation for 6 h, the viability of cells was examined by staining with the live/dead kit (Figure 1 c). Only dead cells were detected by this method, and the biological properties of immobilized (Figure 1 c) and dissolved vancomycin (Figure 1 b) were identical. To test whether immobilized **2** is responsible for the antimicrobial activity, we evaluated the dip-and-rinse solution, the rinsing solution, and a blank incubation solution (the medium without bacteria) for antibacterial activity: All were inactive. These experiments demonstrate that the immobilized hybrid **2** is the active species (see also the leaching experiments discussed below).

We next investigated whether the dead cells remain attached to the functionalized surface, or whether they can be removed by rinsing. Thus, a surface treated with the natural product hybrid **2** was rinsed with PBS buffer (phosphate-buffered saline) after incubation, and the cells were visualized by staining and fluorescence microscopy. Interestingly, a drastic decrease in the number of dead cells was observed (Figure 1 d), and only a small amount of dead bacteria remained on the surface. These results are important, as they demonstrate that the PEG linker displays cell-resistant properties by suppressing the attachment of bacteria to dead cells or cell material. Next, we investigated whether the hybrid **2**, and in particular the vancomycin fragment, is responsible for biological activity.[17] We adsorbed the PEG–anachelin chromophore derivative **1** onto TiO_2 and incubated the resulting PEG-modified surfaces in suspensions of *B. subtilis*. Staining and visualization displayed only living cells and demonstrated no antimicrobial activity for the unfunctionalized PEG derivative **1** (Figure 1 e). Rinsing with PBS buffer again led to a significant decrease in the number of cells on the surface (Figure 1 f). These experiments demonstrate that the conjugate **1** is capable of generating cell-resistant surfaces, but with no antimicrobial activity. This biological property is only observed with the natural product hybrid **2**.

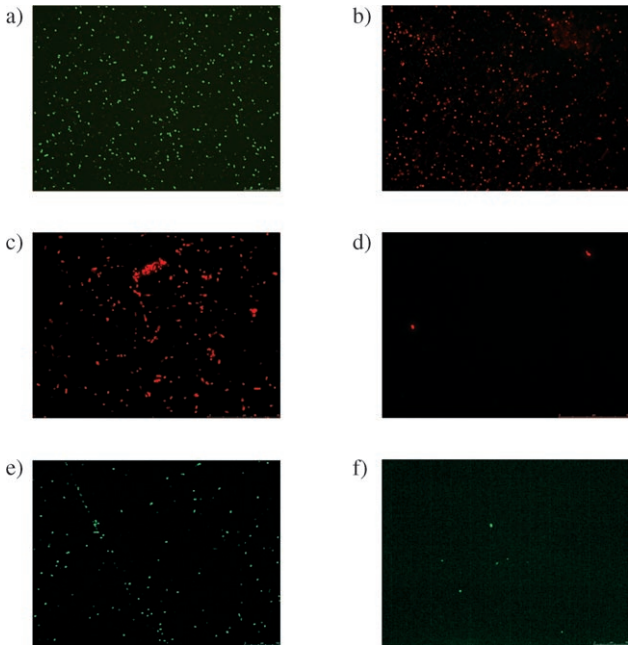

Figure 1. Representative sections of TiO_2 surfaces after incubation in suspensions of *B. subtilis* ATCC 6633 (6 h) and subsequent staining with the live/dead kit. The surfaces were visualized by fluorescence microscopy (see also the Supporting Information): a) untreated TiO_2 surface; b) untreated TiO_2 surface and vancomycin in solution; c) TiO_2 surface modified with **2**; d) TiO_2 surface modified with **2**, after rinsing with PBS buffer; e) TiO_2 surface modified with PEG by treatment with **1**; f) TiO_2 surface modified with PEG by treatment with **1**, after rinsing with PBS buffer.

We investigated whether **2** remains immobilized on the surface upon repeated exposure to a buffer or the medium. Thus, an antimicrobial TiO_2 surface coated with **2** was incubated in a bacterial suspension for 6 h and rinsed with a buffer. This process was repeated five times. Both the antimicrobial activity and the cell-resistant properties remained after five cycles (Figure 2). From these experiments, it can be concluded that the binding of **2** to TiO_2 surfaces is rather strong. This conclusion is corroborated by the results of studies on similar DOPA peptides by other research groups.[18] For example, the DOPA–TiO_2 bond was found to have a dissociation force over 800 pN in AFM studies by Messersmith and co-workers and is thus one of the strongest reversible interactions measured to date.[18d] These investigations underline the great potential of catechols, and in

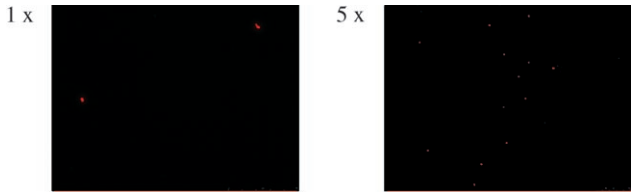

Figure 2. Representative sections of TiO_2 surfaces treated with **2**, after incubation in a suspension of *B. subtilis* and rinsing (left), and after five cycles of incubation and rinsing (right). The surfaces were visualized by fluorescence microscopy after staining with the live/dead kit.

particular of the anachelin chromophore, for the functionalization of surfaces.

Herein, we have presented the synthesis, immobilization, and biological evaluation of the natural product hybrid **2** for the generation of antimicrobial surfaces. This compound combines the properties of the component natural products: The anachelin chromophore enables strong binding to TiO_2 surfaces, and vancomycin is responsible for the antimicrobial activity. Furthermore, the PEG linker contributes to cell resistance; that is, the attachment of dead cells and cell material is suppressed. The benefits of the surfaces modified with **2** include:

1) operationally simple preparation through a dip-and-rinse procedure;
2) strong antimicrobial activity against *B. subtilis*;
3) cell-resistant properties, whereby the attachment of dead cells and cell materials is suppressed;
4) strong surface attachment through the anachelin chromophore and thus high activity after several cycles.

The hybrid strategy described herein should also be applicable to other natural products. In particular, the compatibility of the catechol group with many functional groups found in bioactive small molecules (in contrast to commonly used silanes and thiols) could enable the use of this method for small-molecule microarrays. Furthermore, the unique properties of natural products could be exploited to control biological processes, such as growth, differentiation, and movement, on surfaces through the use of such natural product hybrids.

Received: April 4, 2008
Published online: July 31, 2008

Keywords: antibiotics · biomaterials · conjugates · natural products · siderophores

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