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Inspired by nature: Silencing bacteria

Bacteria love moist surfaces. Once they have settled there, they do not live as loners, but form larger communities that are embedded in a protective film. These so-called biofilms can be found on many surfaces of buildings, in the home on light switches, in the bathroom, on toys or keyboards, in the city on shopping carts or ATMs that many people touch with their hands. This can lead to contact infections. The bacteria, e.g., the pathogenic bacterium *Pseudomonas aeruginosa*, are often persistent and defy the body's own immune system and even biocides or antibiotics. Current research approaches are therefore aiming to prevent or at least make it more difficult for bacteria to colonize materials by modifying their surfaces.

For bacteria to live in communities, it is important that the individual cells "talk to each other". For this purpose, they have developed specific communication pathways and even different "languages". However, communication takes place non-verbally with the help of signaling molecules that are continuously released into the environment. As the concentration of bacteria increases, so does the concentration of the signaling molecules. In this way, bacteria can sense the number of other bacteria in their environment and react accordingly. Once a certain “quorum”, i.e., a certain concentration of signaling molecules or bacteria in the environment, is reached, the individual bacteria activate process chains that lead to the formation of a biofilm. This process is referred to as “quorum sensing” (QS).

Various hosts use the possibility of rendering bacteria "mute" or "deaf" by modifying their signaling molecules or blocking their receptors (docking sites in enzymes). This is done, for example, with the help of haloperoxidases, a group of enzymes that first oxidize halide anions with hydrogen peroxide to form hypohalous acids. The hypohalous acids are then converted to the halogenated signaling molecules. The required halide anions are present in every water film, hydrogen peroxide is formed in sufficient quantities under the influence of UV light.

The halogenated signaling molecules have a similar structure as their non-halogenated counterparts and can therefore bind to the associated receptors. However, they can no longer activate the process chain that leads to the formation of biofilms. The bacteria are thus silenced, a process known as “quorum quenching” (QQ). This prevents bacterial gene regulation from switching to biofilm formation. This interference in bacterial gene regulation is also of pharmacological interest, because pathogenic bacteria can evade the attack of the immune defense or the effect of antibiotics by forming biofilms.

Researchers in Mainz and Koblenz are mimicking this process using nanoparticles of cerium dioxide (CeO_2). CeO_2 nanoparticles, the researchers write in the scientific journal ACS Nano, are a functional substitute for haloperoxidase enzymes and therefore catalyze the formation of halogenated language molecules.

However, the molecular mechanisms underlying biofilm inhibition are difficult to unravel in detail, since not only a plethora of competitive reactions occur in

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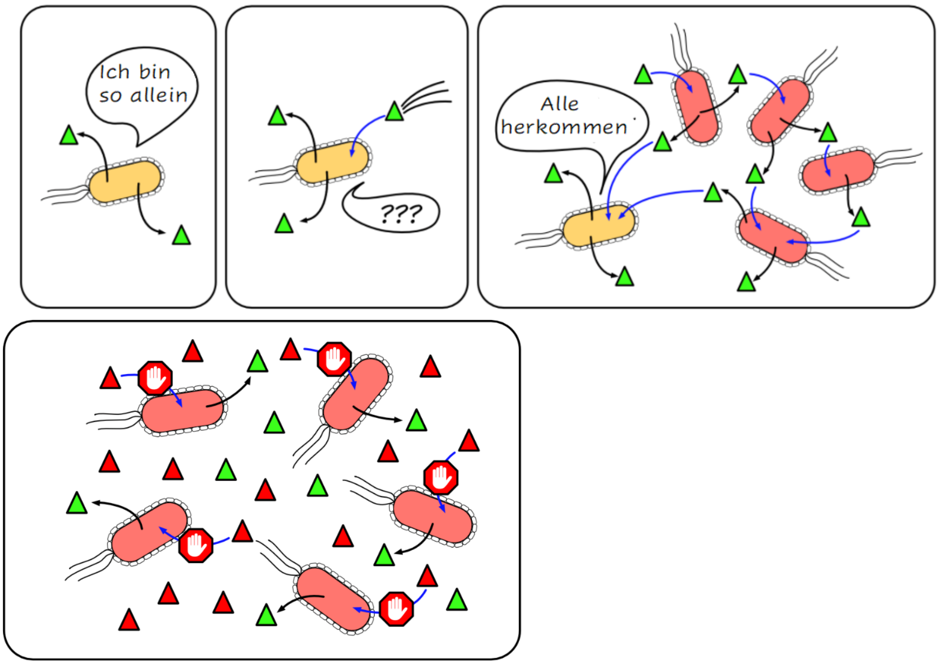
bacterial cultures, but also a vast number of other biomolecules are present in addition to the catalytically modified signaling molecules
(i) Therefore, they first analyzed the molecular reaction cascade triggered when biofilm formation in a culture of P. aeruginosa is inhibited by the enzyme-like activity of CeO_2 nanoparticles. The analysis revealed the catalytic participation of CeO_2 nanoparticles at the molecular level. They inhibit the biofilm formation of several Gram-negative bacteria (*P. aeruginosa, K. pneumonia, M. mesophilicum, P. gallaeciensis*).
(ii) The role of CeO_2 nanoparticles in disrupting bacterial QQ was demonstrated using *Agrobacterium tumefaciens* A136, an engineered biosensor. Gram-negative bacteria such as *P. aeruginosa* use small signaling molecules as QS compounds. CeO_2 nanoparticles catalyze the bromination of some acylhomoserine lactones (AHL), variants of which are specific for different bacterial species. The brominated signaling compounds interfere with QS by either displacing signaling molecules from their corresponding receptor or by covalently modifying and thus inactivating enzymes that produce signaling compounds.

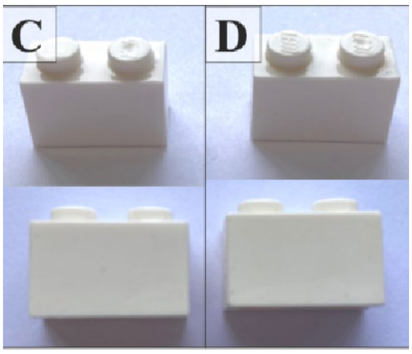
(iii) High-resolution mass spectrometry coupled with liquid chromatography (LC-HRMS) was used to modify halogenated signaling compounds in dispersions containing CeO_2 nanoparticles, H_2O_2, and sodium bromide and to identify the products. However, brominated AHLs could not be detected in bacterial culture fluids of the bacterium *P. aeruginosa* “at work”. Sensitive tandem mass spectrometry coupled with liquid chromatography (LC-MS/MS) also failed to detect them, as the products are presumably rapidly degraded.
(iv) However, via LC-HRMS and a non-target approach (non-target search), the formation of unexpected brominated signaling molecules from the quinolone family could be detected. The formation of these brominated signaling molecules and the inhibition of biofilm formation of *P. aeruginosa* show that CeO_2 nanoparticles interfere with biological processes like native enzymes by inactivating signaling molecules.
A particular advantage is that in the case of undetected infestation with the multidrug-resistant bacterium *S. aureus*, the quinolone signaling molecule was found to lead to the formation of a small colony variant in *S. aureus*, which is often diagnostically undetectable. Infections caused by *P. aeruginosa* and *S. aureus* might be prevented by a suitable coating with CeO_2 nanoparticles.
(v) Finally, the practical applicability of the approach was demonstrated by coating everyday objects with bacteria-repellent transparent polyurethane coatings with embedded CeO_2 nanoparticles. For this purpose, the synthesis of CeO_2 nanoparticles was carried out at the pilot scale. The synthesis route allows the particles to be dispersed in almost any medium (e.g., coatings).
"Cerium dioxide is non-toxic, chemically extremely stable and, for example, contained in modern exhaust gas catalytic converters of vehicles," adds Dr. Eva Pütz, who conducted her doctoral work on this project. She is convinced that cerium dioxide is a viable and cost-effective alternative to conventional biocides. “We have here an environmentally safe component for a new generation of

antibacterial surfaces that mimic nature's defense system. Most importantly, it works not only in the lab but also in everyday use," she adds.
Biofilms are ubiquitous on contact surfaces, e.g. on shopping carts, in buses, on public toilets, on telephones, keyboards or food packaging. The danger in controlling them with biocides and antibiotics is the development of resistance. However, this could be effectively and environmentally friendly circumvented by coating polymers with CeO_2 nanoparticles. In every household in Germany, about 6.1 million tons of food are disposed annually. This corresponds to about 75 kilograms per capita with a value of about 520 €, equivalent to a throwaway rate of about 15 percent. Thus, each German emits on the average about 200 kg CO_2 annually only by food wastes. If only about 40 percent of private disposal could be avoided, up to 7.4 million CO_2 equivalents could be reduced annually. By comparison, an annual speed limit is expected to save only about 1.5 million metric tons of CO_2.

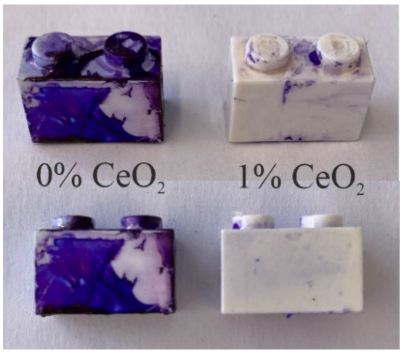
Figures:



Mode of action of quorum sensing. The bacteria (yellow) continuously secrete "signaling molecules" (shown as green triangles). If the signaling molecules are modified (shown as red triangles), the formation of the biofilm is suppressed.
Source: Tremel research group, JGU



Application of CeO_2 nanoparticles (before). LEGO brick commercially treated with polyurethane lacquer (left) and with polyurethane lacquer containing 1% CeO_2 nanoparticles (right)..
Source: Tremel research group, JGU



Application of CeO_2 nanoparticles (after). LEGO brick after incubation in a culture of P. aeruginosa. The bacteria are stained purple. After 72 hours, the bricks treated with lacquer without CeO_2 nanoparticles showed a heavy bacterial coating, whereas the bricks with CeO_2 lacquer did not.
Source: Tremel research group, JGU

Publication:
Eva Pütz, Athanasios Gazanis, Nils G. Keltsch, Olga Jegel, Felix Pfitzner, Ralf Heermann, Thomas A. Ternes, Wolfgang Tremel, Communication Breakdown: Into the Molecular Mechanism of Biofilm Inhibition by CeO_2 Nanocrystal Enzyme Mimics and How It Can Be Exploited. ACS Nano, Online Publication, 30. September 2022; https://doi.org/10.1021/acsnano.2c04377

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