Antimicrobial layer expected to fight hospital acquired infections

Infections caused by bacteria that contaminate the surface of medical devices such as catheters and wound dressings are not that rare and can even be life-threatening. However, at present there is no really effective way to keep these products germ-free until they are used. Scientists at the University of Freiburg have now developed a surface coating that reliably kills bacteria, but is harmless to human cells. The novel surface layer will now be tested in vitro under application-like conditions and validated for subsequent approval.
and becoming increasingly resistant to standard antibiotics. The danger for patients is not only the possible infection route from one person to another, but also infections caused by medical products such as wound dressings and catheters that have been contaminated by bacteria. Such infections kill more than 100,000 people worldwide every year\(^1\). The major problem lies in the so-called biofilms that the bacteria form on the surfaces of medical devices. Biofilms are colonies with countless bacteria that become embedded within a protective slimy matrix. What makes these biofilms so dangerous is that they are resistant to antimicrobial substances and immune system components, and therefore difficult to combat with medical substances.

This is why PD Dr. Karen Lienkamp and her group of researchers at the University of Freiburg have for a number of years been concentrating on ways of keeping the surfaces of medical devices reliably free of bacteria. Lienkamp, who is a chemist by training, initially focused on the development of special antimicrobial coatings. However, this alone was not enough to effectively combat the formation of biofilms. She comments: “Such antimicrobial surface coatings have a positive charge through which the bacteria, whose outer shell has a negative charge, are attracted in the same way as magnets. This kills the first bacterial layer, but is ineffective against those on top of the first one. The coating can be used for devices where only a few bacteria are present. This is why a while ago we started combining such antimicrobial substances with protein-repellent ones. This proved to be much more effective.”

New coating was a chance discovery
To provide the surfaces with a dual activity – an antimicrobial and protein-repellent one – the Freiburg scientists combined short polyoxonorbornene-based zwitterion (PZI) chains with SMAMPs (synthetic mimic of an antimicrobial peptide), artificially synthesised polymers that mimic the structure of natural antimicrobial biomolecules. When they tested the novel surface coating, the Freiburg chemists came across something quite unexpected: the polyzwitterions, which were originally only used as controls, proved to resemble the mythical "egg-laying, milk-bearing woolly sow", as Lienkamp calls her discovery. "We suddenly realised that the polyzwitterions killed off all bacteria on their own and remained protein repellent, i.e. prevented biofilm formation."

In addition, the PZIs proved to be harmless to human cells. The researchers performed toxicity assays on primary and immortalised human cell lines to test this. "The cells grew as well after treatment with PZIs as they did before," explains Lienkamp. "And that's a very good indication that the substance is not toxic. We tested this in solutions and on surfaces."

Paradigm change for polyzwitterions

The polymer will now have to undergo practical testing. Lienkamp and her working group have been awarded funds totaling 1.4 million euros from the German Federal Ministry of Education and Research (BMBF) for their project "ANTIBUG" for a period of three years, which they will use to assess the effectiveness of the novel coating and finish developing it. "We want to show that the coating works as well in practice as it does in the laboratory," says the chemist. "We will also be working closely with industry consultants. For example, we will coat catheters and wound dressings with PZIs and do all sorts of horrible things to them: we will sterilise them using a variety of harsh methods and expose them to tropical conditions in order to ensure storage stability under field conditions and to see if the medical devices are still microbiologically active after such treatments."
The researchers always use several PZIs with different modifications for the tests. “We do this to avoid falling into the trap of just focusing on a single type of coating, which does not have the required stability,” says Lienkamp, explaining her strategy. The amazing polymer has been produced by the group members themselves. Lienkamp says: “We design these structures and subsequently screw them together. This is what we do day in day out. But optimising the stability of polymers is completely new territory for us and is usually neglected in basic research. But this time, things are a bit different. I am tempted to call this a paradigm change as from now on such molecules will be seen in an entirely different way.”

Spin-off or industry cooperation is being planned

In general, all surface coatings tested are thoroughly analysed to find out if they have changed chemically after sterilisation. If they have not, the substances in question are passed on to colleagues from the microbiology department who will investigate whether they still have sufficient antimicrobial activity. Finally, cell biology experiments will be conducted to ensure that the polymer continues to be non-toxic to humans after treatment.

At the same time, the Freiburg scientists are scaling up the synthesis of the polymers: “We are now producing PZIs on the laboratory scale,” explains Lienkamp. “But of course you also have to clarify whether the coating can be produced on a larger scale and still be simultaneously stable and active.” If this works, the PZIs will be synthesised under GMP conditions and all tests will be carried out under certified conditions, such as required for a medical device when marketing authorisation is being sought. “And if everything works as planned under realistic conditions, we may be able to find a pre-cooperation partner over the next three years,” says Lienkamp. “If we manage to do so, we can offer a potential partner reliable data, and this partner will not be buying a pig in a poke.”

Currently, however, the researchers are simply coating two-dimensional substrates with PZIs. “It is difficult to investigate three-dimensional surfaces analytically,” says the scientist. “We will soon start with technical surfaces that we will analyse using fluorine dyes.” Experiments for discovering and developing suitable coating methods will also be carried out. “At present, we use dip coatings, which is what technologists like best. But we will also try many other methods.”
Innovative research at two sites

Theoretically, other applications would also be conceivable in the future, for example coating door handles and light switches. However, Lienkamp prefers to use the polymer for medical applications, at least for the time being. “Bacterial resistances also have to be dealt with. At present, the substance has a relatively low resistance potential, but sooner or later the bacteria will find mechanisms that help them become insensitive to these antimicrobial substances. Antimicrobial substances should only be applied in high-risk areas. Deciding to paint walls with them is no help to anyone. Walls can be cleaned, but it is difficult to clean a catheter.”

The researcher is active at two locations, the Department of Microsystems Engineering (IMTEK) at the University of Freiburg and the Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT). This does not seem to create any difficulties and Lienkamp is quite happy about it. “FIT is a new centre where researchers from a broad range of disciplines, from engineering sciences to biology, work together under one roof. And it is a very inspiring, dynamic location for our projects.”

References:

1 Monika Kurowska, Alice Eickenscheidt, Diana-Lorena Guevara-Solarte, Vania Tanda Widyaya Franziska Marx, Ali Al-Ahmad and Karen Lienkamp: „A Simultaneously Antimicrobial, Protein-Repellent, and Cell-Compatible Polyzwitterion Network“. Biomacromolecules, DOI: 10.1021/acs.biomac.7b00100

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Further information

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