Executive Summary

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Project "Conditional triggered drug release from nanocontainers"

Topic: Oral drug administration is convenient, but has also many drawbacks, such as larger doses, stomach-resistance of drug, systemic drug delivery and thus possible side effects. We are interested in the prevention of implant infections. Implants are generally body-foreign materials without immune system to defend their surface against bacterial adhesion and biofilm formation. Once a biofilm is formed on an implant, it is hard to eradicate with classically administered antibiotics. Hence, researchers have developed antimicrobial coatings for implants, which are most often based on a continuous release of an antimicrobial agent, independently of the presence of bacteria. This leads to a fast consumption of the coating, a permanent flooding of the implant environment with drug and hence to a risk of side effects or even build-up of bacterial resistance.

Goal: To tackle the problem of implant infection, we designed a system allowing to trigger an enhanced antimicrobial drug delivery from the implant surface ONLY if bacteria are present. This design is based on a drug-containing (nano-)capsule to which a bacterial sensor is grafted. Upon a positive sensing event, this sensor triggers an enhanced drug release from the capsule in order to eliminate the bacteria. Such a set-up reduces the amount of required drug, protects it from the biological environment and sets it free only when and where needed.

This project comprised two parts: i) the development of the capsules and their drug release, antimicrobial properties and biocompatibility, and ii) the development of a specific bacterial sensor/trigger system for initiating the drug release. Attaching such a system to an implant surface will then protect the latter from bacterial adhesion and hence implant infection. As the project evolved, we added as goal to develop the sensor as stand-alone product for specific bacterial detection (e.g. for fast colorimetric tests to detect bacterial infections) or for immediate drug release upon bacterial presence without passing by a capsule.

Methods:

1) **Capsule development**: Two methods were used to prepare drug-containing capsules:

a. **Template Method**: First, polystyrene nanospheres are prepared as templates. It is then possible to generate silver nanoparticles (AgNPs) on their surface. These are integrated into the polymer by swelling of the nanospheres. The spheres are then coated with inorganic oxides such as CeO_2, TiO_2 or SiO_2. The polymer core is finally eliminated by either chemical dissolution or by burning away the organic residue.

b. **Microemulsion Method**: A mixture of surfactants and precursors for inorganic oxides (e.g. SiO_2) is used to prepare a reverse water-in-oil microemulsion. A silver salt can be added to the water phase, yielding AgNPs upon reduction. The mineral precursor is then hydrolyzed to form the oxide shell, and to give AgNP-containing nanorattles.

Both methods allow multiple coating steps, with different materials, wall thickness and porosity. All capsules were tested for their amount and duration of Ag^+-release, their antimicrobial properties and biocompatibility. The nanocontainers can be functionalized with fluorescent tags or linkers.

2) **Sensor Development**: Our sensing element is composed of DNA, and acts by hybridization with bacterial RNA and subsequent release of a reactive substrate, which in turn triggers the release of the content of the nanocapsules. A first sensor was based on a functionalized cyclohexane ring, which was kept in a metastable state by the interaction of two partially complementary oligodeoxynucleotides. Upon reaction with the perfectly complementary bacterial RNA, the conformation of the cyclohexane core changes to release a leaving group, enhancing drug delivery from the capsule.

Achievements:
1a) 200 nm sized, AgNP-containing CeO2-nanocapsules were synthesized and are reasonably biocompatible with an antimicrobial Ag+-release over three months for a short to medium protection of implants. A second shell of TiO2 around the AgNP-CeO2-nanocapsules prolongs the release time to an unprecedented ca. 3 years. Enhanced Ag+-release was triggered by a drop in pH or high halide concentrations (leaving groups from sensor). CeO2 and TiO2 nanocontainers were embedded into a TiO2 layer on a metal surface.

1b) 20 nm sized SiO2-nanocontainers and AgNP-filled nanorattles were synthesized. The Ag-filled rattles show good antimicrobial activity against E. coli, S. epidermidis and S. aureus and an exceptional good biocompatibility for dendritic cells, with no immune side effects. The Ag-filled SiO2-nanorattles were used as catalytic reducing agent for methylene blue.

2) The concept of the bacterial sensor was patented as no such system existed before. Several synthetic pathways were pursued to obtain the sensor. The initially planned ester-based sensor was synthesized in small quantities but turned out to be hydrolysis sensitive. A different synthetic strategy allowed the insertion of a linker whose connection to amino-modified SiO2-nanoparticles was successfully tested. A second type of sensor based on a long oligodeoxynucleotide with two functionalized was developed to overcome some of the synthetic difficulties encountered with the first one. This system works successfully within few minutes and at room temperature for Listeria monocytogenes as proven by differential scanning calorimetry and polyacrylamide gel electrophoresis.

Problems: The microemulsion technique 1b) as well as the first sensors of 2) required long times for the fine-tuning of the syntheses and yield only small quantities. To make the sensor-capsule system reversible in terms of the triggering, a further surface-functionalization is required. While working in vitro, the system must also be shown to function in vivo.