Enzyme-responsive nanoparticles and coatings as drug release systems to fight implant associated infections

Henning Menzel, Institute for Technical Chemistry, TU Braunschweig, Braunschweig, Germany, h.menzel@tu-braunschweig.de
Yannick Bourgat & Ingo Krause, Institute for Technical Chemistry, TU Braunschweig, Braunschweig, Germany
Philipp Klahn, Institute of Organic Chemistry, TU Braunschweig, Braunschweig, Germany
Carina Mikolai & Meike Stiesch, Department of Prosthetic Dentistry and Biomedical Material Science, Hannover Medical School and Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE), Hanover, Germany; stiesch.meike@mh-hannover.de

Introduction

Despite significant improvements in the field of biomedical engineering, implant failure due to implant-associated infections is among the greatest medical challenges worldwide. Research activities that aim at inhibiting biofilm formation as the main cause of these infections face several challenges. First, implant surfaces functionalised by static antibacterial layers are only effective when they are in direct contact with the bacteria. Second, drug release systems with a continuous unregulated release of antibacterial drugs that diffuse into the tissue lead to depot depletion after a defined time. Thus, systems in which the release of the drugs is strictly limited to situations of colonisation with pathogenic bacteria would be very attractive. We aim at polymeric systems applied as a coating on implants, which are triggered by bacterial enzymes to release antibacterial agents.

Methods

Suspensions of nanogel particels were prepared by ionotropic gelation of alginate with poly-L-lysine (PLL). PLL in this case was used as a model for more sophisticated peptides with lysine sequences. These peptides as polycations form nanogels with polyanionic alginate by compensating negative charges. The PLL was covalenty conjugated with ciprofloxacin as model antibiotics via copper free click chemistry. The nanoparticles can be applied by spray coating to e.g. titaniums substrates and form homogeneous films. The stability of the nanoparticles in aqous environment with and without addition of enzymes was investigated. The release of ciprofloxacin was monitored. Furthermore, efficacy against S. aureus was tested for the released ciprofloxacin with its conjugated linker residues.

Results

The PLL conjugated with ciprofloxacin formed well defined nanoparticles upon mixing with alginate. The particles have a narrow size distribution and are stable in suspension. They neither agagregate nor were there any indications for a disintegration of the particles or a premature release of the ciprofloxacin. Furthermore, the particles could be easily applied by spray coating to form homogeneous films on titanium as examples for an implant material. Upon addition of enzyme the PLL is degraded resulting in a disintegration of the particles and release of the ciprofloxacin. The films were also degraded upon addition of enzyme. The released ciprofloxacin bears remains of the linker groups, thus the antibiotic efficacy was tested. Indeed it was found that the efficacy is affected by the acylation at the piperazin ring of the ciprofloxacin with sterically very demanding bicyclononatriazole-ring system.

Conclusion

Alginate-based nanoscale hydrogel particles were prepared as a drug delivery system that releases the drug triggered by the presence of an enzyme. The nanoparticles are stable in suspension and can used to prepare coatings. Both the nanoparticles and the coatings were stable, but degraded by the addition of an enzyme, and the active ingredient was released. This approach was successfully tested with ciprofloxacin as the antibiotic drug and poly-L-lysine as the model for a peptide. The influence of the linker on the activity of the ciprofloxacin was tested. It was established that the voluminous groups of the linker residue bound to the ciprofloxacin via an acyl bond reduce the antibiotic activity.Thus we are now developing new self-immolative linker groups which release the antibiotic drug without any remains.

Single Cell Force Spectroscopy to Analyze Bacterial Adhesion on Implant Materials

Katharina Doll, Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Hannover, Germany, doll.katharina@mh-hannover.de
Andreas Winkel, Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Hannover, Germany, winkel.andreas@mh-hannover.de
Ines Yang, Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Hannover, Germany, yang.ines@mh-hannover.de
Meike Stiesch, Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Hannover, Germany, stiesch.meike@mh-hannover.de

Introduction

A profound understanding and improved characterization of the initial bacterial adhesion is essential to further reduce the development of bacterial biofilm-caused infections of medical devices like implants. Single cell force spectroscopy using atomic force microscopy is a versatile technique for this purpose. However, analyses of single cell adhesion forces are not yet available for most bacterial species.

Methods

In the present study, the adhesion of several clinically relevant bacteria of the highly diverse oral microbiome to the implant material titanium was analyzed by means of microfluidic-assisted force spectroscopy on a single cell level. The maximum adhesion force, number of attachment points and the detachment distance were quantified under two environmental conditions. Furthermore, potentially contributing cellular parameters such as cell elasticity, membrane integrity, and metabolic activity were examined to gain a deeper insight into the adhesion process.

Results

This study provides the first adhesion force spectra for several oral bacterial species. Measured forces corresponded to species-specific niches in oral biofilms: pioneer colonizers exhibited highest values, followed by pathogenes and commensal secondary colonizers with the lowest. It could be shown that bacterial initial adhesion forces depend mainly on the charge of surface molecules and less on other factors such as cell rigidity, intact membrane, or active metabolism.

Conclusion

The study provides new insights into fundamental processes of bacterial adhesion to implant materials. Therefore, it will contribute to the development of innovative approaches to prevent microadherence-associated problems in medicine.

New Biocompatible Materials for Active Stimulus-responsive Dental Implants

Christian Klose, Institut für Werkstoffkunde (Materials Science), Leibniz University Hannover, Germany, klose@iw.uni-hannover.de
Philipp-Cornelius Pott, Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Germany, pott.philipp-cornelius@mh-hannover.de
Hans Jürgen Maier, Institut für Werkstoffkunde (Materials Science), Leibniz University Hannover, Germany, maier@iw.uni-hannover.de

Introduction

Treatment of peri-implantitis is one of the major challenges in modern dentistry. In this context, dental implants that will provide a tailored local application of antibiotics appear promising. Compared to the state of the art, the envisaged active stimulus-responsive implants however require higher-strength materials to compensate for the larger cavity that must accomodate both the antibacterial agent as well as a miniaturized pumping mechanism. With niobium-zirconium (Nb-Zr), a promising alloy free of potentially toxic constituents is available as an implant material that can reach very high strength. This material also shows pronounced osteoconductivity.

Methods

Samples were machined out of rectangular Nb-Zr billets that underwent severe plastic deformation using the equal channel angular extrusion method. The mechanical properties of the ultrafine-grained (UFG) Nb-Zr specimens were determined in 3-point bending tests and compared with titanium grade 4 as well as zirconium dioxide that are used in dentistry as materials for crown and bridge frameworks as well as implant-fixtures and implant-suprastructures. Basic tests to assess the adhesion of gingiva fibroblast cells to Nb-Zr samples were also conducted.

Results

The results of the mechanical testing showed that the average maximum bending forces of UFG Nb-Zr (818 MPa ± 56 MPa) were lower than for titanium (1066 MPa ± 17 MPa), but generally above those of ZrO_2 (621 MPa ± 186 MPa) and zirconia with additional regeneration-sintering (723 MPa ± 151 MPa). In addition, the deformation of both metals, titanium (3633 µm ± 207 µm) and UFG Nb-Zr (1726 µm ± 97 µm), at maximum bending force was considerably higher compared with both types of zirconia (186 µm ± 62 µm and 159 µm ± 30 µm, respectively). In the cell tests, a decent accumulation of fibroblast cells on the surfaces of both Nb-Zr and titanium samples was observed.

Conclusion

It can be concluded that UFG Nb-Zr is a promising material for dental implants, especially due to the combination of its good load-bearing capacity and biocompatibility. Clearly, the strength of UFG Nb-Zr must be improved by alloy modification and an adjustment of the manufacturing process in order to compensate for the planned reduction in wall thickness that will be necessary to integrate the micro pump into the implant body. In this context, preliminary studies showed that a substantial increase in hardness of UFG Nb-Zr can be achieved by selective oxidation of the Zr in the alloy.

Sensory cochlea electrode through detection of critical processes at the electrode nerve interface.

Martin Durisin, Hals-Nasen-Ohrenklinik und Poliklinik, Medizinische Hochschule Hannover, Hannover, Germany, durisin.martin@mh-hannover.de
Thomas Lenarz, Hals-Nasen-Ohrenklinik und Poliklinik, Medizinische Hochschule Hannover, Hannover, Germany, thomas.lenarz@mh-hannover
Kirsten Wissel, Hals-Nasen-Ohrenklinik und Poliklinik, Medizinische Hochschule Hannover, Hannover, Germany, wissel.kirsten@mh-hannover
Sabine, Behrens, Institut für Werkstoffkunde, Leibniz Universität Hannover, Hannover, Germany, sbehrens@iw.uni-hannover.de
Hans Jürgen Maier, Institut für Werkstoffkunde, Leibniz Universität Hannover, Hannover, Germany, maier@iw.uni-hannover.de

Introduction

Cochlear implants (CI) have undergone enormous technical development, particularly in the areas of speech coding, processing and signal transmission. However, platinum corrosion of cochlear implant electrodes can lead to implant dysfunction resulting in poor speech understanding and loss of implant control. In addition, the product of platinum corrosion, i.e., dissolute containing platinum ions, may have a cytotoxic influence on different cell lines. The aim of the study was therefore to investigate the effects of platinum dissolute on cells.

Methods

Platinum dissolute was obtained by electrical stimulation of electrode contacts and was added to different cell lines. The metabolic activity of the model cells, i.e., murine fibroblasts (NIH 3T3) and the human neuroblastoma (SH-SY5Y) cell line was determined using the WST-1 assay.

Results

At specific concentrations, Pt-dissolut led to mitochondrial swelling in both cell types indicating cytotoxicity. As demonstrated by TEM, platinum was internalizedby specific cell types. TEM imaging also revealed both mitochondrial disintegration and swelling of the endoplasmic reticulum, suggesting that Pt ions trigger cytotoxicity in both NIH 3T3 and SH-SY5Y cell lines by interacting with the respiratory chain.

Conclusion

The results show significant cytotoxicity mediated by dissoluted platinum. Thus, developing a fail-safe CI by detecting critical processes at the electrode-nerve interface is an unmet clinical need and overall goal of the this project. A chronic stable electrical stimulation of neural cells ist essential for hearing by cochlear implants. A corrosion resilience of the stimulation electrode is one of the essentials for achieving this goal. To this aim, the electrodes themselves could be used to detect changes in the phase boundary state. The detection of these changes could be determined by using cyclo-voltammetry and electrochemical impedance spectroscopy.

Simulation of multie-species bacterial coaggregation in dental implants

Meisam Soleimani, Institute of Continuum Mechanics, Leibniz university Hannover, Hannover, Germany, soleimani@ikm.uni-hannover.de

Szymon Szafranski, Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Hannover, Germany, Szafranski.Szymon@mh-hannover.de

Meike Stiesch, Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Hannover, Germany, stiesch.meike@mh-hannover.de

Peter Wriggers, Institute of Continuum Mechanics, Leibniz university Hannover, Hannover, Germany, Wriggers@ikm.uni-hannover.de

Introduction

Implants are broadly utilized in numerous medical disciplines as the replacement of missing biological structures in order to restore quality of life. Here the focus is on the dental implants that are constantly exposed to indigenous oral microorganisms that readily form complex multi-species biofilms. Peri-implant biofilms disrupt the proper function of the implant and lead to chronic infections that are severely resilient to antimicrobial treatment. This work deals with the mathematical modeling of bacterial coaggregation and its numerical implementation in an FEM framework. Since the concept ”coaggregation” refers to the cooperation between multi-species of bacteria, a system composed of two species is considered in the modeling framework. The extension of the model to arbitrarily more species is straightforward.

Methods

A phase-field modeling approach is employed to describe the growth of both species as well as the coaggregation between them. The model is 3D and fully based on the continuum description of the problem without any need for discrete agents which are the key elements of the individual-based modeling approach. The details of numerical implementation in an FEM framework are also presented. Indeed, a new multifield user element is developed in ANSYS for this multiphysics problem. Predictions of the model are compared with the experimental observations and the versatility and applicability of the model and the numerical tool are well established.

Results

Several numerical examples are presented to show the robustness of the proposed numerical tool in handling multi-species biological systems. In order to understand the spatio-temporal structure of such bacterial community one should identify and understand the interaction, partnership, and communication among the members of this community. The results are promising in the sense that the proposed mathematical model is caple of capturing such interactions between different species of biofilms. The predictions made by the numerical tool can be improved through validating the *in silico* results against *in vitro* observations. Apart from the development of methods for the rapid simulation of biofilm dynamics, our findings will facilitate the tailored design of implants with effective capabilities of biofilm prevention and control. It will, in the future, contribute to a significant increase in implant safety.

Conclusion

The overarching aim of this work is to develop a practical and robust framework for combating peri-implant biofilms. It consists of two complementary, mutually supportive aspects: *in vitro* investigations and *in silico* simulations. Numerical simulation techniques are applied to model biofilm formation and degradation in the presence of antimicrobial agents. The ultimate goal is to select the most promising anti-biofilm drug candidates and it will be validated in organoids or animals and, in the longer perspective, translated into clinical applications.