Functional Nanogel Coatings as Antifouling and Antibacterial Surfaces

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General Introduction & Aim of this Thesis

1.1 **Biomaterial-Associated Infections**

Biomedical implants and devices have made a great impact on patient health for decades. The use of hip and breast implants, voice prosthesis, artificial heart valves, stents, urinary catheters, vascular grafts, and dental implants is rapidly growing.[1] However, the risk of an implant-associated infection always comes along with the biomaterials that could end up in a complete malfunctioning of the implant.[2] After insertion of the implants into the body, if a free-swimming (planktonic) bacterium attaches to the biomaterial surface, it may start the bacterial colonization process. Subsequently, the biofilm begins to grow with complex and dynamic structures while initiating an extracellular matrix formation (Figure 1).[3,4] If not treated on time, this process can end up with an acute infection, causing implant failure and requiring replacement surgery with high costs.[5] Biofilms have lower susceptibility towards antimicrobials and host defense mechanisms when compared to planktonic bacteria and therefore are challenging to treat.[6] Thus, preventing the initial bacterial attachment and further biofilm formation is crucial to avoid implant-associated infections and implant failures.

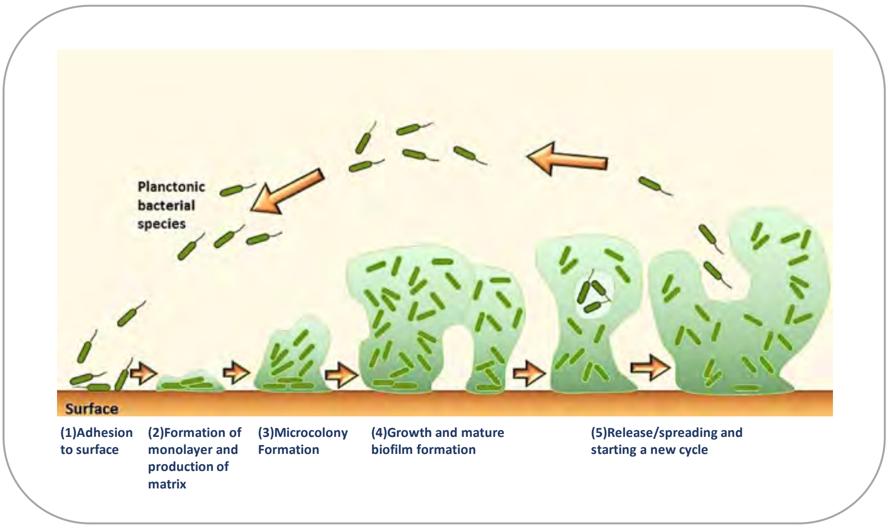

Figure 1. Schematic representation of a biofilm formation. (1) Initial planktonic bacteria adhesion to the surface. (2) Bacteria form a monolayer and irreversibly attach by producing an extracellular matrix. (3) Next, a microcolony is formed with multilayers. (4) The biofilm is formed. (5) Finally, some cells start to detach, and the biofilm will disperse.[7] (Reprinted with permission from MDPI)

1.1.1 **Antibiotic Resistance**

According to the World Health Organization, bacterial infections with subsequent biofilm formation represent one of the biggest global threats to human health since conventional antibiotic therapies gradually become ineffective because of antibiotic resistance.[8,9] The vast overuse of antibiotics in medical treatment and increased travel and migration stimulates the spread of multidrug-resistant bacterial strains.[10] Antibiotic-resistant bacterial strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE) are some examples of antibiotic-resistant bacterial strains which can be found in implant and biomaterial-associated infections, causing serious concern in healthcare services.[11] Therefore, new and effective approaches are urgently needed to prevent and control biofilm infections.

1.1.2 **Alternative Approaches for Preventing Biofilm Formation**

The traditional clinical attempt to solve the infection problem is the administration of antibiotic drugs. The design of antibacterial coatings has become an alternative approach, which is strongly required due to the ineffectiveness of antibiotics' traditional administration.[12] Lately, anti-adhesive surface coatings have gained much interest to inhibit the initial bacterial attachment on the surface as an alternative platform to control biofilm formation and subsequent infections. For instance, surface modifications can be performed by changing the surface chemistry or topography using polymer-based hydrogels, zwitterionic polymers, or various polymer brushes, which are able to resist microorganism adhesion.[13–15] As another approach, contact-killing coatings are often employed, which is created by confining antimicrobial molecules at biomaterial surfaces, and bacterial killing is supposed to occur due to the disruption of the cell membrane. Quaternary-ammonium compounds (QAC) are very well-known antimicrobial molecules that are used to prepare contact-killing surfaces.[16] Additionally, release-based coatings are also advantageous when compared to traditional antibiotic methods. The direct localized release of an antimicrobial substance from the material surface offers the delivery only where needed, thus preventing toxicity from reaching other parts of the body and minimizing resistance development.[17] However, the development of new antimicrobial coating alternatives is still needed. In this thesis, a new antiadhesive and killing approach will be presented as surface coatings prepared with hydrogel-based nanoparticles, so-called "nanogels," to help diminish future multi-resistant bacterial strains and biomaterial-associated infections.

1.2 **Hydrogel Materials**

Hydrogels are three-dimensional cross-linked systems consisting of a hydrophilic network that can swell and entrap a large amount of water within its network while maintaining the structure due to chemical or physical cross-linking of individual molecules or polymer chains.[18] The biocompatibility and flexibility of hydrogel-based materials, which is due to their significant water absorption capability, offer possibilities for utilizing hydrogels in numerous applications such as contact lenses, surface coatings, membranes, and drug delivery platforms.[19–21]

1.2.1 **Polymeric Nanogels**

Polymeric nanogels, or nanogels in short, are nanometer-sized hydrogel nanoparticles with three-dimensionally cross-linked polymer networks.[22] They also have a remarkable ability to absorb high volumes of water or biological fluids similar to conventional hydrogels while maintaining their structure due to the presence of cross-links between the hydrophilic groups in the polymer matrix. Their biocompatibility is attributed to high water content;

meanwhile, they also demonstrate excellent colloidal stability.[23] These soft and smart polymeric nanoparticles have the possibility to display a stimuli-responsive behavior to environmental triggers such as temperature, pH, and ionic strength when synthesized using the appropriate monomers.[24] Moreover, nanogels can be modified to incorporate biomolecules or to provide a wide variety of chemical functionalities.[25] On top of that, their mechanical properties can also be altered by tuning the internal cross-linking density.[26]

1.3 **Nanogel Coatings for Biomedical Applications**

Nanogels can be used to modify surfaces and thereby create coatings as an alternative approach to avoid biomaterial-associated infections.[27] These smart nanoparticles offer robust and facile approaches to create well-organized surface coatings while giving possibilities to incorporate different chemical functionalities to be used for various purposes.[28,29] Nanogel-based coatings have been assembled via different deposition techniques such as dip coating, spin coating, or spray coating.[30] They can be covalently grafted or physically adsorbed on the surface, forming monolayer or layer-by-layer arrangements.[31] When the aim is to design new surfaces of nanogel coatings for any desired application, it is crucial to have a homogeneous and uniform particle assembly on the surface.[32] One of the most used processes is based on the adsorption of charged nanogels onto the surfaces via electrostatic interactions. Nanogel properties, nanogel-surface interactions, and nanogel deposition techniques are some of the parameters that affect the formation of homogeneous monolayer or multilayer structures and a good surface coverage with a close-packed assembly.[33,34] By increasing the understanding of these parameters, we would be able to control the final properties of the assembled nanogels on the surface, as well as their further functionalization.

Nanogels in dispersion show much potential as drug delivery systems in particular due to their drug loading capacity, encapsulation stability, water solubility, biocompatibility, and responsive behaviors.[35] Also, surface-bound nanogels offer a wide range of opportunities for biomedical applications due to the unique properties of nanogels. Nanogel coatings have been attractive for many years for various applications such as drug delivery systems, biosensors, cell adhesion and proliferation models, protein and macrophage repellent surfaces.[36–39] Nanogel coatings are more advantageous than more complicated synthetic procedures (e.g., surface-initiated polymerization), as the nanogel properties are easily controllable. Therefore, nanogels provide a platform to design surfaces with desired features such as thickness, mechanical properties, porosity, and functionality.[40–42]

As described earlier, conventional antibiotic usage is ineffective to combat biomaterial-associated infections; therefore, novel and practical methods are currently needed to avoid and combat biofilm infections. In this context, nanogel coatings offer a promising solution to inhibit the initial bacterial adhesion on the surface to avoid the problem in the early phases of biofilm formation.[40] Moreover, bacterial killing properties can also be introduced by the functionalization of nanogels, which is another vital strategy to prevent infections on the biomedical implant surface.

1.4 Aim of this Thesis

The general aim of this thesis is to explore hydrogel and nanogel coatings as novel antifouling and antibacterial systems to inhibit bacterial adhesion and growth on the biomedical implant surfaces, focusing on surface-bound nanogels with various functionalities. Thereby, the general aim consists of three sub-aims: (i) the synthesis, functionalization and characterization of nanogels in bulk solution, (ii) the preparation of the coating with synthesized nanogels on the surface, coating characterization and modification, (iii) the evaluation of bacteria-repelling and bacteria-killing performances of nanogel coatings.

The overall envisioned outcome of this project is a new platform for performing a simple and reliable coating design and optimization of the nanogel coating processes to be potentially used in the biomedical field to prevent biomaterial-associated infections. (Figure 2).

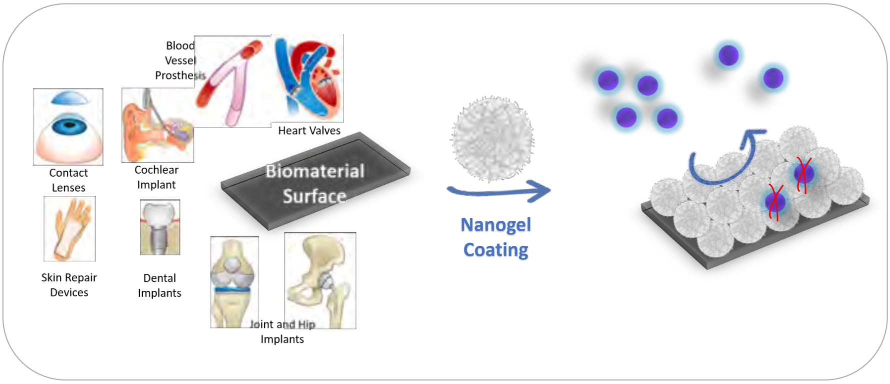

Figure 2. A schematic summary of the aim of this thesis. Antifouling and antibacterial nanogel coatings as an alternative platform to prevent bacterial infections on the biomaterial surfaces.

1.5 Structure of this Thesis

This thesis consists of six chapters. **Chapter I** gives a basic overview of the biomaterial-associated infection problems and highlights the motivation and the aim of this research.

Chapter II discusses the current state-of-art in the field of nanogels and nanogel coatings in terms of efficiency and applicability for combating infections in the biomedical area, including implant-associated infections, which are addressed.

Chapter III presents a straightforward approach to preparing a hydrogel coating on silicone rubber, and it also provides insights into the notable drawbacks. The relation between the hydrogel coating features and the mechanical properties of the substrate provides a more in-depth insight into how the biomaterial itself can be affected by applying a coating and supports the need for exploration of alternative hydrogel-based coatings such as the use of nanogels.

In **Chapter IV,** the antifouling performance of nanogel coatings of different thickness and stiffness was evaluated.

Chapter V further demonstrates how the functionalized nanogels and nanogel coatings can be used to enhance antimicrobial performances by incorporating antimicrobial strategies combined with antifouling properties to have a complete bacteria-repelling and bacteria-killing surface.
The overall nanogel-based coatings offer many possibilities, but some drawbacks may still arise, and future development should be placed in proper perspective, which is discussed in **Chapter VI.**

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