

DSM Non-Biofouling Coatings: A stealth approach to the fighting biofilms.

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Abstract

DSM has developed and markets a variety of hydrophilic UV curable coatings. These hydrophilic coatings include ComfortCoat™ lubricous coatings and (biocidal) antimicrobial lubricous coatings for both catheters and guide wires, as well as newly developed VitroStealth™ non biofouling coatings. In this paper we will highlight the technological and clinical benefits of our entire UV coatings platform and principally the novel non-biofouling technology. For our Non-fouling coatings we will demonstrate dramatic reduction in protein adsorption and cellular (microbial and blood) adhesion.

Introduction:

Non-fouling synthetic surfaces are highly desired for the development of biocompatible medical implants, biosensors and a diverse range of non-biomedical applications. The notion 'non-fouling' is generally used to characterize surfaces that, in contact with a biological fluid, resist unintended accumulation of biological entities, which may lead to, for example, the formation of a biofilm. Upon exposure of a synthetic surface to biological media, the initial event is the rapid adsorption of macromolecular moieties, forming a so-called conditioning film for the subsequent adhesion of other molecular and cellular entities¹⁻⁵. A number of studies, following various strategies, have focused on the surface modification of materials to prevent the initial and non-specific adsorption of macromolecular components, notably proteins, biological cells and microorganisms.

One approach to render surfaces non-fouling, is the adsorption or covalent grafting of hydrophilic polymer chains or oligomer with brush-like structures. The most widely utilized polymer for achieving non-fouling properties and improved biocompatibility both in-vivo and in-vitro is poly(ethylene glycol) (PEG)⁶. A number of strategies for preparing brush-like PEG surfaces involve for example, adsorption of block copolymers with a non-adsorbing and a strongly adsorbing block, cross linking of star-shaped PEG polymers, end-grafting of reactive PEG chains, and transferred Langmuir-Blodgett films⁷⁻¹¹. However, the majorities of these methods are substrate-specific and require tailored steps of surface preparation and therefore a generally applicable grafting method is desirable.

In the aforementioned studies, the desired interfacial properties were attained by a monomolecular layer grafted to a substrate, yielding coatings that are susceptible to

mechanical damage, resulting in a localized loss of non-fouling performance. Thereby, the use of functional monolayers in everyday applications is limited and there is an ongoing need to develop coatings that combine the desired surface functionality of densely grafted monolayers with mechanical and (bio)-chemical robustness.

In this paper we present the results of a study pertaining to the inhibition of protein adsorption and cellular adhesion on robust coatings with a high density of grafted hydrophilic polymers. The coating formulations consist of colloidal silica nano-particles with reactive acrylate groups and hydrophilic PEG chains, suspended in a methanol – water mixture. After application of the coating and evaporation of the solvent, the particles are cross-linked by exposure to ultraviolet (UV) radiation.

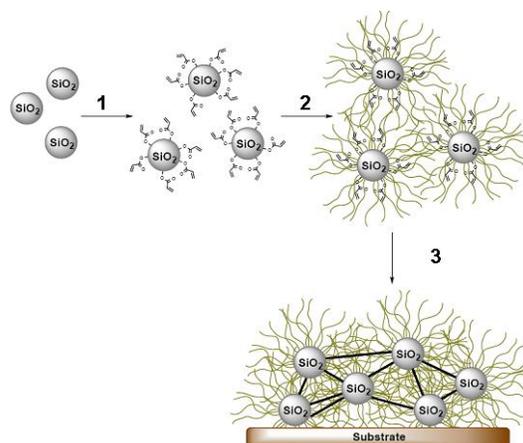


Figure 1. Schematic representation of silica oxide nano-particles surface modified with acrylate groups (Step 1), grafted with mPEG chains (Step 2) and followed by coating application and photopolymerization (Step 3).

Upon contact of the cross-linked coating with water, the PEG chains at the surface become hydrated and swell, providing a brush-like structure at the surface, as can be seen in figure 1.

Methods

Protein Adsorption Measurements

Protein adsorption on the coated Si/SiO₂ surfaces was measured in real-time using stagnation point flow reflectometry¹². The change in intensity of the reflected polarized He-Ne laser following adsorption on the spin-coated silicon wafer was converted into adsorbed mass using a 5-layer matrix model.^{15,26, 27} The coatings were immersed in a 10 mM sodium nitrate solution for at least 10

min to equilibrate. Lysozyme (Aldrich, MO, Lot 51K7028) (0.156 mg/mL) was dissolved in the same sodium nitrate solution (pH 6.2, 250 mL). Human blood plasma was diluted 100 times using PBS buffer at pH7. All experiments were carried out in triplicate at a flow rate of 1.0 mL/min. After introduction of the sodium nitrate solution to the flow chamber for at least one min, the protein solution was allowed to flow into the chamber. Once an adsorption plateau had been reached, the sodium nitrate solution was flushed through the chamber to replace the protein solution. This procedure was then repeated once allowing investigation of protein adsorption to a surface previously exposed to the protein.

Bacterial Strains

Staphylococcus epidermidis HBH 276 were used in this study. Colonies were streaked on a blood agar plate and grown overnight at 37°C. Both strains were maintained as frozen stock kept at 4°C for no longer than 2 weeks. A colony was used to inoculate a 10 mL tryptone soya broth (TSB; Oxoid, UK) and was incubated at 37°C for 24 h in ambient air. This preculture was used to inoculate a second culture of 200 mL, and allowed to grow for 16 h at 37°C. Bacteria from this second culture were harvested by centrifugation (5 min at 10,000g) and washed twice with ultrapure water. Subsequently, bacteria were resuspended in 10 mL phosphate-buffered saline (PBS, 10 mM potassium phosphate, 150 mM NaCl, pH 6.8) and sonicated for 10s on ice. Finally, the bacterial suspension was diluted in 200 mL of PBS to a concentration of 3×10^8 mL⁻¹.

Bacterial Adhesion Measurements

Adhesion of bacteria on the coated glass slides was monitored in a parallel plate flow chamber (internal dimensions 175 x 17 x 0.75 mm³). Bacterial deposition and subsequent detachment can be directly observed using phase contrast microscopy. Bacteria adhering to the glass slides were observed with a CCD-MXRi camera (High Technology) mounted on a microscope (Olympus BH-2) equipped with a x 40 ultra long working distance objective (Olympus ULWD-CD Plan 40 PL). PBS and bacterial suspensions were circulated through the chamber by hydrostatic pressure at a flow rate of 1.5 mL/min⁻¹, corresponding to a Reynolds number of 0.6 and a wall shear rate of 10 s⁻¹. The bacterial suspension was allowed to flow for 4 h and images were collected via a dedicated image analyzer (TEA; Difa). Each image (512 x 512 pixels with 8 bit resolution) was obtained after summation of 15 consecutive images (time interval 1 s) in order to enhance the signal to noise ratio and to eliminate moving bacteria from the analysis. A single image corresponds to a surface area of 0.0096 mm² and computer software was used to count the number of adhering bacteria. Each data point is an average of two independent images. All experiments were carried out in

triplicate with separately cultured bacteria and newly prepared substrata.

Results

The non-fouling performance of the resultant coating towards proteins was investigated by stagnation point reflectometry using a wide variety of proteins of various size and charge, including human blood plasma, see figure 2. For the optimized coatings protein adsorption was found to be at detection limit for all tested proteins.

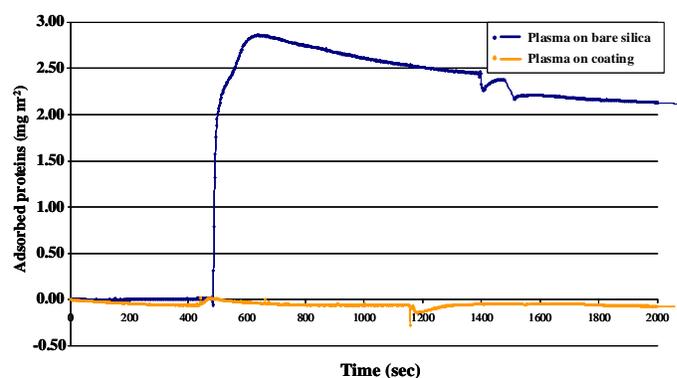


Figure 2. Protein adsorption results obtained from stagnation point reflectometry for human blood plasma (100x diluted) on bare silica (blue) and on the non-adhering coating (orange).

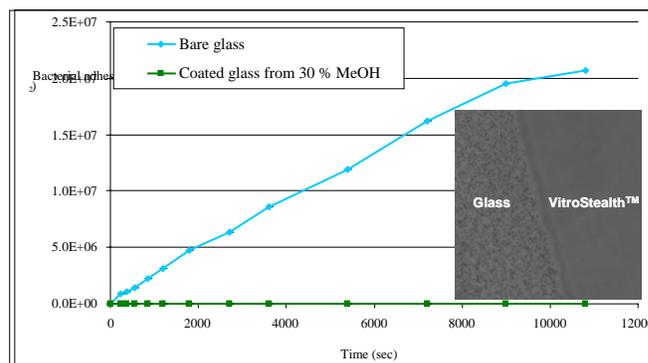


Figure 3. Adhesion of *Staphylococcus epidermidis* (HBH 276) under flow conditions a) Number of bacterial cells per square centimeter as a function of flow time for both uncoated glass (blue) and on the VitroStealth™ coated glass (green) Inset) Micrograph of *S. epidermidis* adhering after 4 hours of flow.

As stated previously the adhesion of cellular entities such as for example bacteria in biofilm begins with the formation of a conditioning layer of macromolecular species (proteins) onto which the cells subsequently adhere. Thus by preventing the adhesion of proteins we should be able to prevent the adhesion of cells also. This is indeed the case for bacteria such as *Staph. Epi.*, see figure 3. After ca 3 hours of flow untreated glass displays around 2×10^7 bacterial cells per square centimetre while on the non-fouling coated glass no bacterial adhesion at all was observed under the same flow conditions. Similar non-adhesive behaviour has been observed for human (blood) cells and this will be discussed

in more detail. Furthermore the mechanical and physical properties of the coatings will be discussed in more detail.

Discussion

Our non-fouling coatings platform combines state-of-the-art non-biofouling properties, such a dramatic reduction in protein adsorption and cellular adhesion, with remarkable mechanical durability¹³ and very low extractables. As such we believe that they will form a valuable tool in medical device design, leading to improved performance, reliability and most importantly patient outcomes.

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