

## DSM Science & Technology Awards 2004

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## Summary

This thesis introduces novel surface coatings for biological and medical applications derived from six arm star shaped prepolymers. These polymers consist of statistically copolymerized ethylene oxide and propylene oxide in the ratio 4:1 as backbone and bear reactive isocyanate endgroups (star PEG) introduced by functionalization of OH terminated stars with isophorone diisocyanate.

Preparation of the star PEG coatings on aminosilanized silicon wafers or glass samples by spin casting from aqueous THF solutions results in smooth and homogeneous films as monitored by optical microscopy and by scanning force microscopy. The intrinsic tendency of the system to dewet the substrate is thereby chemically quenched by crosslinking of the star shaped prepolymers. This process results in a dense network of poly(ethylene glycol) (PEG) chains connected via urea groups with a well defined meshsize of the network determined by the molecular weight of the star molecules and hence by their armlength. Due to the high functionality of the star molecules, not all isocyanate groups participate in the crosslinking reaction and free amino groups remain in the layers that have been detected by fluorescence microscopy and by XPS.

Film thicknesses can be varied specifically by altering the concentration of the solution prior to spincoating or by changing the rotation speed during spincoating. The resulting layers exhibit a strong contact angle dependence on the layer thickness that originates from the amphiphilic character of the star prepolymers with hydrophilic backbones and hydrophobic endgroups. The system preferentially arranges the hydrophobic endgroups at the air-polymer interface to create a surface with minimal surface energy. This is possible in absence of water or in thick films, since in both cases the crosslinking which quenches the ordering process is slow. In aqueous environment the films are rearranging towards a surface with maximal hydrophilicity. This contact angle hysteresis increases with increasing layer thickness and is reaching values of  $90^\circ$  for very thick films.

Films thinner than 50 nm prepared from water/THF mixtures in the ratio 9:1 show a contact angle hysteresis less than  $30^\circ$  and exhibit excellent protein resistant properties. The unspecific adsorption of avidin, streptavidin, lysozyme, insulin and ribonuclease H was analyzed by fluorescence microscopy, surface MALDI-TOF MS and single molecule spectroscopy and was found to be neglectable. In comparison experiments, the star PEG layers even perform better than linear PEGs and physisorbed denaturated BSA as surface, two systems widely used as anti adhesive surface modifications.

## *Summary*

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Furthermore, layers between 15 and 30 nm thickness prepared from water/THF 9:1 prevent the adhesion of mouse MT3C3 cells as well as the adhesion of SaOS cells, human skin derived keratinocytes, human skin derived fibroblasts and human mesenchymal progenitor cells (hMPC).

Besides glass and silicon, the star PEG layers have been applied successfully to aminofunctionalized titanium substrates and amino-ppx coated silicon wafers. The coating of aminosilanized PDMS resulted in star PEG films that showed crack formation with time. As alternative approach, preliminary experiments with ammonia plasma treated PDMS show the formation of homogeneous and stable star PEG layers that prevent protein adsorption.

The star PEG layers can easily be statistically modified with water soluble, isocyanate reactive compounds by adding them to the water phase before mixing with the star prepolymer solution in THF prior to spincoating. Thereby, biocytin has been embedded into the layers, and biotinylated ribonuclease H was immobilized on the layers via streptavidin. For FRET experiments, a donor/acceptor pair of Alexa 546/647 dyes was attached to the ribonuclease H. Chemically induced unfolding and refolding was, in contrast to linear grafted PEG and denaturated physisorbed BSA as surfaces, reversibly possible on the star PEG layers over 50 cycles. Thermodynamical evaluation furthermore proved that the folding process is thermodynamically only neglectably influenced by the surface, underlining the minimal interaction of the surface with the immobilized proteins.

The oligopeptide gRGDsc has been covalently embedded into star PEG layers following the same procedure. The nonadhesive nature of the films could thereby be changed towards specific cell adhesion capability. SaOS cells, human skin derived fibroblasts and hMPC could be cultivated on these layers for several weeks. Furthermore, differentiation of the hMPC into osteoblast like cells could be chemically induced on the layers and proved by RT-PCR. This shows that the star PEG coatings do not possess cell toxic properties and do not influence the differentiation potential of progenitor cells.

The star PEG layers can also be modified after layer formation by microcontact printing ( $\mu$ CP). By stamping on crosslinked layers with an amine reactive biotin derivative, streptavidin could be immobilized in patterns. The anti adhesive properties of the unmodified star PEG layer in between the biotin modified pattern yielded a very good signal to noise ratio.

Stamping on freshly prepared star PEG layers that still contain isocyanate groups allows covalent attachment of aminofunctional compounds. Thereby, nitrilotriacetic

acid (NTA) groups, protected by ester groups, were patterned onto star PEG layers. Deprotection of the NTA groups was achieved thermally and wet chemically, but wet chemical treatment was found to be superior. Due to the changes of the mechanical properties of the layers with crosslinking, the pattern obtained from one stamp can be varied by either stamping directly after layer preparation or one hour later. The obtained NTA patterns can be loaded with Ni(II) ions and are capable to specifically bind His-tag proteins. This was visualized by immobilization of His-tag EGFP. The protein repellent properties of the unmodified star PEG coating in between the NTA pattern and the specific NTA/His-tag recognition even enabled immobilization of His-tag EGFP in the pattern by immersion of the sample in crude His-tag EGFP containing cell lysate.