Cellular interactions with chemical gradient surfaces

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SUMMARY

During the last 10 years, gradient surfaces have evolved into popular tools for the study of protein adsorption and cellular interaction phenomena with solid surfaces (see **Chapter 1** for a literature survey). The introduction of gradient surfaces by Elwing (1986) was stimulated by several problems that occur within this field of research. The interaction of proteins and cells with solid surfaces are mediated by several surface parameters like wettability, specific chemistry and surface roughness. In many studies, several materials with widely varying surface chemistries are used and the interpretation of the results is often difficult because other parameters like wettability and roughness are varied simultaneously. In addition these studies are also time consuming and biological variations are more likely to occur. The use of gradient surfaces reduces these problems. On a gradient surface one chemical property is continously varied along a specific direction of the surface. Gradient surfaces are profitable in studying the basic mechanisms by which complicated systems such as proteins or cells interact with surfaces, since a continuum of selected and controlled physical-chemical properties can be studied in one experiment on the surface.

In **Chapter 1**, a literature survey of currently employed methods to prepare and characterize gradient surfaces is given. Furthermore, their application for the study of protein adsorption and cellular interaction phenomena is discussed. In general proteins adsorb more extensively on the hydrophobic ends of gradient surfaces, which is accompanied by a lesser spreading and adhesion of tissue cells than on the hydrophilic ends of gradient surfaces. An influence of the specific chemistry, constituting the gradient, can always be found both on protein adsorption as well as on cellular interactions.

In **Chapter 2**, a model was developed for the variation in surface chemical heterogeneity along the length of various n-alkyl chain monochlorosilanes gradients on glass, based on advancing and receding water contact angle measurements and scanning X-ray photoelectron spectroscopy. Data from both methods were used independently of each other to calculate the surface fraction covered by n-alkyl silane which increased gradually when going from the hydrophilic side toward the hydrophobic side. A main difference from both techniques was that the fraction of the surface covered by n-alkyl silane was higher over the entire length of the gradient according to water contact angles than by XPS. It was concluded

that water contact angles are more sensitive to changes in surface chemical heterogeneity along the length of chemical gradient surfaces than X-ray photoelectron spectroscopy.

In **Chapter 3**, the adhesion, spreading and distribution of human skin fibroblasts on dimethyldichlorosilane gradient surfaces on glass was investigated. In the presence of serum proteins, human skin fibroblasts seeded on these gradient surfaces showed a preferential adhesion onto the steepest part of the gradient, probably due to an optimal local wettability and/or local chemistry. Furthermore it was shown that the spread area of human skin fibroblasts increased over the length of the gradient surface when going from the hydrophobic to the hydrophilic end.

In **Chapter 4**, human umbilical vein endothelial cells were seeded onto steep and shallow dichlorodimethylsilane gradient surfaces on glass substrates and exposed to fluid flow in a parallel plate flow chamber. After 3 hours of adhesion and before onset of the flow, cells adapted relatively large spread areas on the hydrophilic side of the gradient surfaces as opposed to cells on the hydrophobic side which were small and almost circular. After onset of the flow, cells detached immediately from the hydrophobic side of the gradients. Apart from these general observations, an influence of both wettability steepness and direction of flow on cellular distribution and shear induced migration was found.

In **Chapter 5**, human umbilical vein endothelial cells were seeded onto dichlorodimethylsilane (DDS), dimethyloctadecylchlorosilane (DOCS) and (tridecafluor-1,1,2,2-tetrahydrooctyl)-1-dimethylchlorosilane (TFCS) gradient surfaces on glass and exposed to fluid shear stress in a parallel plate flow chamber. On DDS gradient surfaces, cells withstood flow best in regions with a wettability gradient with advancing water contact angles around 25 degrees while on DOCS and TFCS gradient surfaces this optimum was located around 65 degrees advancing water contact angle. The importance of the underlying specific chemistry is shown by the fact that alkyl chains on DOCS gradient surfaces and fluorocarbon chains on the TFCS gradient surfaces, induce cellular adhesion to be optimal at higher water contact angles compared to cellular adhesion on DDS gradient surfaces which are constituted by relatively small methyl groups.

In **Chapter 6**, growth, spreading and shape of human skin fibroblasts and human umbilical cord endothelial cells on dichlorodimethylsilane (DDS) and dimethyloctadecylchlorosilane (DOCS) gradient surfaces was investigated in the presence of

serum proteins. Endothelial cell growth was fully inhibited on the hydrophobic side of the DDS gradient surface, but not on the hydrophobic side of the DOCS gradient surface. In contrast, spreading of both fibroblasts and endothelial cells during growth was approximately uniform over the length of DDS and DOCS gradient surfaces. By comparison with studies involving only adhesion and spreading of cells in the absence of growth, it is suggested that exchange interactions between adsorbed serum proteins and endogeneous adhesive proteins are responsible for cell spreading during growth on the hydrophobic sides of the gradient surfaces.

In **Chapter 7**, the general discussion to this thesis, the advantages and disadvantages of the use of gradient surfaces for the study of protein adsorption and cellular interaction phenomena are considered. Furthermore, suggestions are raised for future studies with gradient surfaces, such as *in vivo* studies, microbial adhesion studies and the preparation and application of "roughness" gradient surfaces instead of chemical gradient surfaces.

Summarizing, this thesis demonstrates that gradient surfaces are valuable research tools for the study of protein adsorption and cellular interaction phenomena. Gradient surfaces offer the opportunity to determine the influence of, for instance, surface wettability on cellular interaction phenomena in one single experiment, therewith avoiding possible biological variations which can occur over longer periods of time. In addition major influences of widely varying surface chemistries on cellular interactions are avoided since gradient surfaces are caused by a gradual change of one chemical property.