INTERACTION OF HEPATOCYTES WITH FOREIGN SUBSTRATA

Thomas Groth [1] and George Altankov [2]

[1]*Institute of Chemistry, GKSS Research Centre, Teltow, Germany,* [2] *Institute of Biophysics, Bulgarian Academy of Sciences, Sofia, Bulgaria*

INTRODUCTION: One promising approach for the treatment of acute hepatic failure is the extracorporal liver support system employing isolated or transformed hepatocyte immobilised on polymer membranes. One of the basic problems is still the long term-maintenance of large amounts of viable and metabolically active cells during the application of the device. A better understanding of hepatocyte-surface interactions could help in the development of polymer membranes with improved compatibility for hepatocytes. In this paper we have investigated the interaction of human hepatoblastoma cells with hydrophilic and hydrophobic glass and different polymer membranes. We tested how the properties of the underlying substratum determine the interaction with hepatocytes and learned about the interplay between attachment and morphology with growth and function.

METHODS: Clean and octadecylsilane (ODS) coated glass were used as models for hydrophilic and hydrophobic surfaces, respectively. C3A cells - a hepatoblastoma cell line, and freshly obtained porcine hepatocytes were cultured for a short-time period up to 7 days on the above substrata. Further experimental work was carried out with polymer membranes made of moderate wettable polyacrylonitrile (PAN), poly(acrylonitrile-N-vinylpyrrolidone) P(AN-NVP), polyvinylidendi-fluoride (PVDF) and hydrophobic polyetherimide (PEI) membranes. Materials were characterised by water contact angle measurements. The initial attachment of hepatocytes was studied, then focal adhesions were stained with anti-vinculin, actin by FITC-phalloidin and cell-cell contacts by E-cadherin antibodies and visualised immune-fluorescence. Cell proliferation was measured by LDH-assay and functional activity by secretion of albumin.

RESULTS & DISCUSSION: We could show that both C3A cells and normal porcine hepatocytes, spread better on hydrophilic glass than on ODS. Spreading was accompanied by the development of pronounced actin stress fibres and focal adhesion contacts. In contrast, on hydrophobic substrata predominant cell-cell interactions took place, which led to intense E-cadherin staining in the intercellular contacts of porcine hepatocytes but not in C3A cells. On the other hand metabolic activity and growth of C3A cells were reduced on hydrophobic ODS, but albumin synthesis was not different on both surfaces. Investigations with polymer membranes showed that C3A cells attached slowly and tended to make aggregates. Interestingly, initial adhesion at 2 and 4 h tends to follow the general rule that cells adhered less efficiently on hydrophobic surfaces since adhesion was lower on the PEI membrane in comparison to the more wettable PAN and P(AN-NVP) membranes. On the other hand, P(AN-NVP) membranes had a reduced initial attachment, up to 8 h incubation, which might be explained with the ability of this copolymer to take up larger quantities of water. The results from actin and vinculin staining show that cells growing on PAN possessed mostly longitudinal organised cytoskeleton and developed a higher number of focal adhesion complexes. Although growing in aggregates, the cells in the periphery were more spread than on the other membranes, which correlated with the moderate wettability of the PAN membrane. However, a quite similar morphology of C3A cells was observed on the hydrophobic PEI membrane. Although actin was more condensed (in the middle of aggregates) the hepatocytes looked well spread and developed sufficient amount of focal adhesion contacts, in comparison to cells grown on the relatively hydrophilic P(AN-NVP) and PVDF membranes. While the morphology of cells was quite different on these membranes, proliferation was almost the same. Interestingly a close correlation was found between the formation of cell-cell contacts and the functional activities of cells in terms of albumin secretion. Particular NVP as most hydrophilic material provoked homotypic adhesion with low proliferation and high secretory activity of cells.

CONCLUSION: Materials with moderate adhesivity provoking homotypic adhesion such as hydrogel-like surfaces may be applicable as substrata for the immobilisation of hepatocytes if sufficient amounts of cells are seeded.

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