Leibniz Universität Hannover
Institut für Technische Chemie, Callinstr. 3, 30167 Hannover
Partner in der Forschung

Polysialic acid from E.coli K1 as bio-identical material for cell cultivation and tissue engineering applications

Yvonne Stark, Stephanie Bruns, Bastian Rode, Frank Stahl, Cornelia Kasper, Thomas Scheper
Institut für Technische Chemie der Universität Hannover, Callinstr. 3, 30167 Hannover

Introduction

The polysialic acid with its non-immunogenic properties and biocompatiblity is an promising component for cell cultivation and tissue engineering. Polysialic acid (PSA) is a dynamically regulated posttranslational modification of the neural cell adhesion molecule (NCAM). NCAM-PSA acts as important regulator in the development of brain structures and in processes accompanying learning and memory. Some neuroinvasive bacteria like *Escheria coli K1* or *Neisseria meningitidis serogroup B* are encapsulated by PSA and the capsule provides an important virulence factor. This capsular polysaccharide is identical to PSA found in the human body. The aim of this study was to compare different established cell culture coatings like coll I and poly-l-lysine with PSA. As reference β-glucan, and uncoated tissue culture plastic were investigated. The modified cell culture surface- cell interactions were studied using model cell lines HepG2, PC-12 and immortalised Schwann cells (ISC).

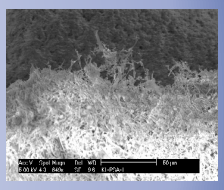

Figure 3: SEM-pictures of K1 polySia ;650x

Materials and methods

Cell culture:
PC-12 cells were cultured in DMEM, 10 % HOS, 5 % FCS, L-glutamine, Na-pyruvate and antibiotics.
HepG2 cells were cultured in DMEM containing 10 % NCS and antibiotics.
IS cells were cultured in DMEM containing 10 % FCS, L-glutamine, Na-pyruvate and antibiotics.
Cell metabolism: Cell viability was assayed using **MTT-test.**

Coating procedure and cell seeding:
The wells of 96-well plates were covered with 40 µl of each solution and incubated for 1 h at room temperature and 30 min at 6 °C. Plates were washed twice with PBS and seeded with 4000 cells (HepG2 and PC-12) and 3000 ISC per well, respectivly

Control samples

Tissue culture plastic: Uncoated tissue culture plastic refers to the unmodified surface of multi-well tissue culture plates.

PLL: 5 mg/ml *poly-L-lysin* was dissolved in ddH_2O.

Collagen I:1mg/ml collagen I from calf skin was dissolved in 0.1 N acetic acid and stirred 3 h at room temperature. The solution was dissolved 1: 4 with ddH_2O.

Colominic acid: 5 mg of colominic acid (CA) (purchased) was dissolved in 1 ml ddH_20.

β-glucan: 5 mg β-glucan was dissolved in 1 ml NaOH 1 N.

Polysialic acid: 5 mg of PSA (isolated from *Escheria coli K1* by our group) was dissolved in 1 ml ddH_20.

Results of colominic acid

First experimentes were performed with the commercial colominic acid (CA). The CA was tested in its soluble form and compared to other coating materials, which are in use for cell culture already. Hence, the focus of this study was to determine the effect of polysialic acid on the cells compared to traditional extracellular matrix factors such as collagen and poly-l-lysine. Additional materials like the polysaccharide β-glucan and uncoated plastic cell culture surfaces were tested as references.

The cell viability results for all cell lines showed increasing viability on all used materials during the cultivation. The colomininc acid showed no cytotoxic effect. Therefore, further experiments could be performed with the polysialic acid isolated .

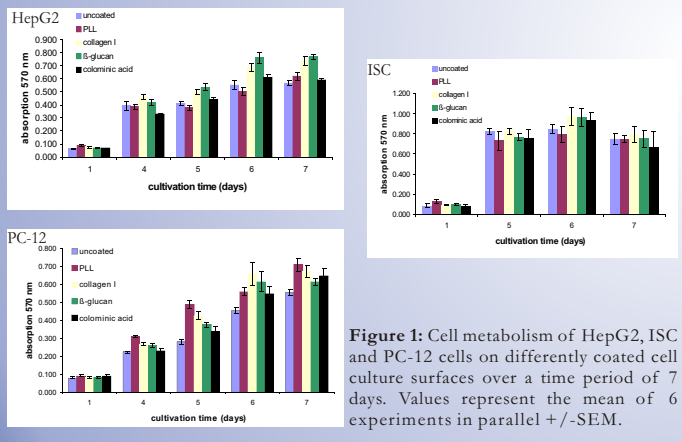

Figure 1: Cell metabolism of HepG2, ISC and PC-12 cells on differently coated cell culture surfaces over a time period of 7 days. Values represent the mean of 6 experiments in parallel +/-SEM.

Results of polySia by E.coli K1

The following experiments were done with PSA isolated of *E.coli* K1 which produces the human like PSA of α-2,8-linked 5-N-acetyl-neuraminic acid

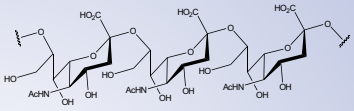

Figure 2: Human PSA of α–2,8-linked 5-N-acetyl-neuraminic acid (colominic acid)

During the cultivation PSA is released to the medium. Subsequent to harvesting, bacteria and supernatant were separated by continous centrifugation. The polysialic acid in the supernatant was concentrated by ultra-filtration and separated from the contamination by fractional precipitation with ethanol. The raw product was desalted by dialysis and freeze dried.

The freeze dried polysialic acid was dissolved in dH_2O and plates were covered with the solution. After 1h the plates were washed and PC -12 and IS cells were settled on the coated surfaces. Via MTT-Test the cell growth was observed on the PSA coated surfaces. As reference commercial colominic acid and uncoated cell culture surfaces were used.

Both cell lines grow on the PSA-K1 and CA coated surfaces. The viability increases over the whole cultivation time. The cell viability showed a similar course on all the materials. The PSA-K1also showed no toxic effeco.

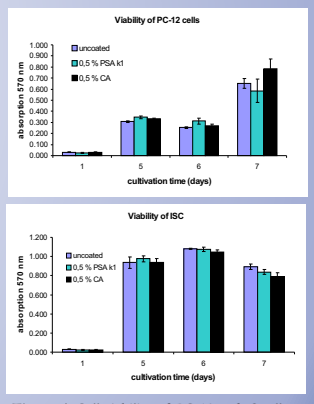

Figure 4: Cell viability of PC-12 and IS cells on PSA-K1, CA and uncoated surfaces.

Conclusion and Outlook

The results of the cell viability assays show that the cells are viable on all tested materials. All used cell lines grow on all differently coated cell culture surfaces. In comparison to the established coating materials and the uncoated surface on the commercial CA equivalent values could be reached. Also the comparison of the CA and the PSA-K1 results in a similar course of the cell viability. The CA and the PSA-K1 do not show cytotoxic effects. In this study the applications of polysaccharide polysialic acid as putative biomaterial for cell cultivation was demonstrated. These were the first results for PSA testing in its soluble form. In the future the production of the PSA-K1 will be optimised to reach higher yields. For cell culture experiments a crosslinking will be performed and insoluble modified materials will be tested .

Deutsche Forschungsgemeinschaft DFG

Acknowledgement

This research work was performed with the DFG Forschergruppe 548 "Polysialinsäure Evaluation eines neuen Werkstoffs als Gerüstsubstanz für die Herstellung artifizieller Gewebe" supervised by Prof. R. Gerardy-Schahn. We like to thank Prof. R. Gerardy-Schahn for her excellent support during this work.

DFG Forschergruppe 548 Polysialinsäure polysia