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Comparative testing of surface coatings for cell culture applications

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Introduction

One of the main objectives for cell cultivation and tissue engineering is the choice of precise scaffold material in order to support and guide cell growth and cell differentiation. Therefore, numerous materials, fabrication techniques and modifications have been used and tested recently to fulfil different requirements depending on the cell type. But so far, technology is far from optimized and there is great demand of new or modified biomaterials suitable for cell culture applications. Promising materials must be tested in suitable cell culture systems prior to application for tissue engineering. In this study a comparative screening of different biomaterials towards their applicability for cell culture applications was established.

In the present study, polysaccharids (polysialic acid (PSA) and β-glucan) are tested in its soluble form as coating material with regard to further application as scaffold, especially PSA for nerve regeneration. We compared these polysaccharides to different established cell culture coatings like collagen I, poly-L-lysin,and hyaluronic acid. These investigations were the first promising steps in our ongoing research regarding the production and application of PSA and β-glucan as scaffold materials. Model cell lines HepG2 and PC12 were seeded on the different coated plates and cultured over a time period of one week. Within the presented screening the cell morphology, viability and adhesion were analysed and compared with the differently coated plates. For β-glucan a crosslinking step was accomplished and the toxicity of the first modified material was tested additionally.

Materials and methods

Cell culture:
Pc12 cells were cultured in DMEM, 10 % HOS, 5 % FCS, L-glutamine, Na-pyruvate and antibiotics. Cells can be differentiated with DMEM supplemented with 1 % HOS, 1 % FCS, 1 % L-glutamine, 1 % Na-pyruvate, antibiotics and NGF (100 ng/ml).
HepG2 cell were cultured in DMEM containing 10 % NCS and antibiotics.

Cell metabolism: Cell viability was assayed using **MTT-test.**

Cell adhesion assay: Cell adhesion was determined by DAPI-staining of the cells after 2 h and 24 h.

Neurite extension assay: For neurite extension measurement 40 000 cells were seeded in 24 well plates for each coating system. The length of more than 40 neurities were measured and the mean value was determined. In addition the number of cells with neurites was counted.

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 per well.

Control samples

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

PLL: 5 mg/ml PLL was dissolved in ddH_2O.

Collagen: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.

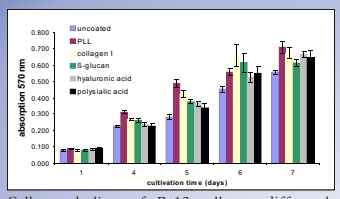
Hyaluronic acid: 3.5 mg hyaluronic acid (HA) was dissolved in 1 ml ddH_2O

Polysialic acid: 5 mg of polysialic acid was dissolved in 1 ml ddH_2O.

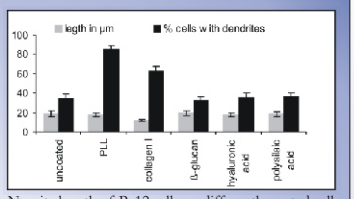
β-glucan: 5 mg β-glucan was dissolved in 1 ml NaOH 1 N.

β-glucan gel SB 01/03: β-glucan was dissolved in NaOH 1 M and incubated with different diepoxyoctan concentrations for 24 h. The gels were washed with aceton and dialysed for 5 days. After desinfection with isopropanol it could be used for the experiments.

Cell metabolism and differentiation of Pc12 cells

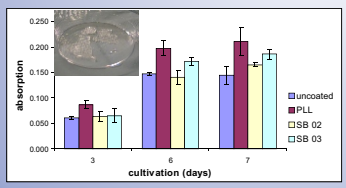


Cell metabolism of Pc12 cells on differently coated cell culture surfaces over a time period of 7 days. Values represent the mean of 6 experiments in parallel +/-SEM.



Neurite length of Pc12 cells on differently coated cell culture surfaces after 3 days differentation and number of neurite extending cells.

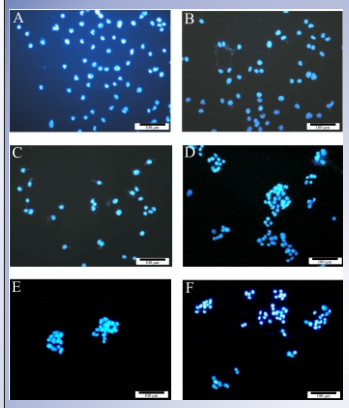
Cell viability results of PC12 cells showed increasing viability of the cells on all used materials during the cultivation. PSA and β-glucan indicated no toxic effects. Cell adhesion showed a similar course on all used materials and equivalent cell numbers were observed after counting the cell nuclei for each experimental condition (data for adesion not shown, comparable to HepG2 cells). PC12 cells were successfully differentiated on all used materials. Equivalent neurite length were observed.



Cell viability of PC-12 cells after addition of different modified β-glucan hydrogels over a time period of 7 days. Values represent the mean of 6 experiments in parallel +/-SEM.

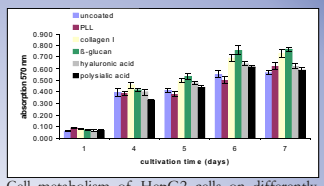
After crosslinking of β-glucan with different concentrations of diepoxyoctan a hydrogel was established. The toxicity of the hydrogel was also tested by MTT-assay. A small piece of the gel was added to the cell culture medium. PC12 cells showed an increasing viablity over the cultivation time of 7 days.

Cell metabolism of HepG2 cells

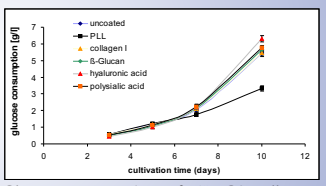


DAPI staining of HepG2 cells on differently coated cell culture surfaces after 24 h. A: uncoated, B: poly-L-lysin, C: collagen I, D: β-glucan, E: hyaluronic acid, F: polysialic acid.

HepG2 cells attach on all materials. Equivalent cell numbers were observed after counting of the cell nuclei for each experimental condition. In figure D, E, F cells tended to build agglomerates. For PC12 cells comparable results were observed.



Cell metabolism of HepG2 cells on differently coated cell culture surfaces over a time period of 7 days. Values represent the mean of 6 experiments in parallel +/-SEM.



Glucose consumption of HepG2 cells on differently coated cell culture surfaces over a time period of 7 days. Values represent the mean of 6 experiments in parallel +/-SEM.

The cell metabolism assay and glucose consumptions of HepG2 cells on differently coated cell culture surfaces showed a fast cell growth on all materials during the cultivation. PSA showed no toxic effects. Similar cell viability and glucose consumption levels were achieved on all used materials.

Conclusion and Outlook

The results of the different assays (viability and cell adhesion) show, that the cells are viable on all used materials. Both cell lines grow and attach on all differently coated cell culture surfaces. In comparison to the established coating materials and the uncoated surface on PSA and β-glucan equivalent values could be reached. In this study the application of both polysaccharides β-glucan and polysialic acid as putative biomaterials for cell cultivation was demonstrated. These were the first results for PSA testing in its soluble form. In the future a crosslinking will be performed and insoluble modified materials will be tested as done for β-glucan.

Acknowledgement

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