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A Study on Biopolymers for Tissue Engineering Applications with Regard to Nerve Regeneration

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Introduction

In this study, polysialic acid and collagen I were tested for tissue engineering with regard to peripheral nerve regeneration. Colominic acid (CA) is a homopolymer of sialic acid residues and is solely composed of polymerised units of α-2,8-linked N-acetylneuraminic acid. CA is a specific derivative of polysialic acid (PSA), produced as the capsular polysaccharide of E.coli K1 derived molecule of PSA. It 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.

In the present study, the cytotoxicity of the polysaccharides and their influences on viability were observed. Additionally, the colominic acid was crosslinked with diepoxyoctan to form a hydrogel. The modified cell culture surface - cell interactions were investigated using the model cell lines HepG2 and PC-12. Additionally Matristypt®, a collagen I basis material, was evaluated and characterised. Moreover, different cell markers were tested by RT-PCR studies.

Results and Conclusions

Cytotoxicity studies on colominic acid

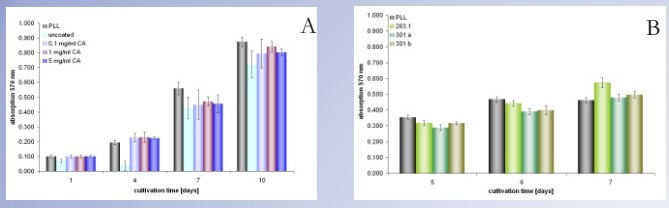


Figure 3: Cytotoxicity of PC-12 cells after addition of different CA (0.1-5 mg per ml medium) concentration (Figure A) and after addition of differently crosslinked hydrogels (Figure B). Values represent the mean of 6 experiments in parallel +/-SEM.

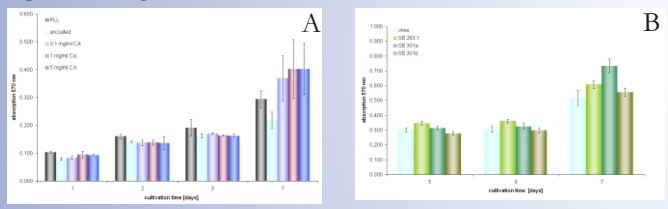
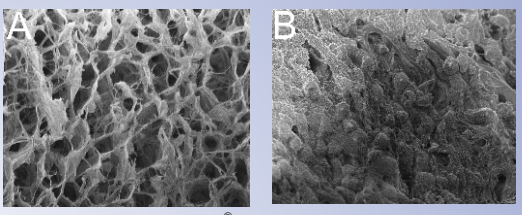


Figure 4: Cytotoxicity of Hep-G2 cells after addition of different CA (0.1-5 mg per ml medium) concentration (Figure A).and after addition of differently crosslinked hydrogels (Figure B). Values represent the mean of 6 experiments in parallel +/-SEM.

Studies on collagen I



A: SEM micrograph of Matristypt® without cells. (magnification 400x) B: SEM micrographs of Matristypt® seeded with ISC and cultivation over 14 days. (magnification 1000x).

The collagen I matrix without cells shows a porous structure. After settling the matrix with cells and cultivation over 14 days the complete matrix is covered with cells.

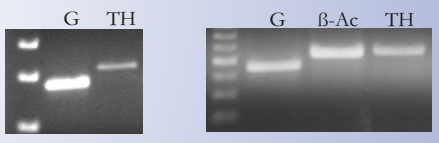


Figure 5: RT-PCR of ISC cells and PC-12 cells on Matristypt®: G: GAPDH, 452 bp;
ß-Ac: beta-Actin, 536 bp;
TH: Tyrosin Hydroxylase, 596 bp

The porous structure of the collagen biomaterials seems to be very suitable since the ISCs grow in a dense cell layer on these materials. The RT-PCR results showed the expression of the specific cell marker tyrosine hydroxylase and the house keeping genes GAPDH and beta-Actin on the collagen material Matristypt®. Approximatly all PC12 cells express thyrosine hydroxylase on Matristypt®, which was also confirmed by immunocytochemistry. Moreover the colominic acid showed no cytotoxic effect on the cells. High viabilities were observed over the culitvation time.

Summary and Outlook

The results of the cell viability assays indicate that the cells are viable in the presence of colominic acid(CA) and their hydrogels. PC-12 and HepG2 cells reached high viabilities after addition of different CA and hydrogels concentrations. Polysialic acid and particularly its specific derivate the colominic acid could be interesting basic materials for cell culture applications and should be continued to characterised. The modification of the materials to hydrogels was the first step and further experiments will be performed to test the materials in more detail. The collagen I matrix, Matristypt®, is also an interesting basic material for tissue engineering applications. The results showed a regular distribution of the ISC on the material surface. Different cell makers like the tyrosine hydroxylase and the house keeping genes were expressed.
The combination of collagen I with its suface structure, and colominic acid, wich plays a significant role in synaptic plasticity and neural development, could be be formed to interesting materials with regard to peripheral nerve regeneration.

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