(P 320) Production of Recombinant Carbohydrate—Binding Modules Fused To RGD: Functional Studies using Bacterial Cellulose

F.K. Andrade[1], S.M.G. Moreira[1], L. Domingues[1], F.M.P. Gama[1]

[1]IBB—Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, 4710-057 Braga Portugal.

The attachment of cells to biomedical materials can be improved by using adhesion molecules, present in the extracellular matrix substances, such as fibronectin, vitronectin, or laminin. In many cases, Arg-Gly-Asp (RGD) was found to be the major functional amino acid sequence responsible for cellular adhesion. In the present study, a method for producing chimerics proteins, RGD-CBM, with functions similar to fibronectin, which contains a cellulose-binding module (CBM), was developed. The CBM used was from the cellulosoma of the bacteria Clostridium thermocellum. The genes encoding these CBM-containing chimeric proteins were cloned, and the protein expressed and purified. Bacterial cellulose (BC) secreted by Gluconacetobacter xylinus was produced. Polystyrene surfaces and bacterial cellulose sheets where "coated" with these RGD-containing proteins, and then used in adhesion/biocompatibility tests, using a mouse embryo fibroblasts culture. The results showed that the proteins containing the RGD or GRGDY sequence were able to improve the adhesion of the fibroblast on the polystyrene plate, furthermore proteins containing the RGD sequence were more effective than the proteins containing the GRGDY sequence. Preliminary adhesion studies of fibroblast cultures on cellulose sheets, functionalized with the recombinant proteins, showed positive effects on the adhesion and proliferation of the cells. The results demonstrated that the proteins containing the RGD sequence were able to increase significantly the adhesion of fibroblast to BC surfaces when compared with the controls (cellulose treated with the CBM or buffer). The results also demonstrated that the protein containing one RGD sequence have a stronger effect than the protein containing two RGDs.