The Effect of an RGD-Human Chitin binding domain fusion protein on the adhesion of fibroblasts to reacetylated chitosan films

Vera Carvalho, Lucília Domingues, Francisco Gama

IBB – Institute for Biotechnology and Bioengineering, Centre for Biological Engeineering. Universidade do Minho, Campus de Gualtar 4710-057 Braga, Portugal

Abstract

Biomaterials used for tissue engineering applications must provide a structural support for the tissue development and also actively interact with cells, promoting adhesion, proliferation and differentiation. To achieve this goal, adhesion molecules may be used, such as the tripeptide Arg-Gly-Asp (RGD). RGD was found to be the major functional amino acid sequence responsible for cellular adhesion. This sequence can be used to elicit specific cellular responses and it has been extensively demonstrated that RGD sequence improves cell adhesion, spreading and proliferation in different materials. Chitosan and chitin represent a family of biopolymers, made up of β(1→4)-linked N-acetyl-D-glucosamine and D-glucosamine subunits. Due to their biodegradability and biocompatibility, chitin and chitosan, are widely studied for biomedical applications.

A method based on the use of a human Carbohydrate-Binding Module, with affinity for chitin, was tested as an alternative approach to the chemical grafting of bioactive peptides. This approach would simultaneously allow the production of recombinant peptides (alternatively to peptide synthesis) and provide a simple way for the specific and strong adsorption of the peptides to the biomaterial. A fusion recombinant protein, containing the RGD sequence fused to a human chitin-binding module (ChBM), was expressed in *E. coli*. The adhesion of fibroblasts to reacetylated chitosan (RC) films was the model system selected to analyse the properties of the obtained proteins. Thus, the evaluation of cell attachment and proliferation on polystyrene surfaces and reacetylated chitosan films, coated with the recombinant proteins, was performed using mouse embryo fibroblasts 3T3. The results show that the recombinant proteins affect negatively fibroblasts anchorage to the materials surface, inhibiting its adhesion and proliferation. We also conclude that this negative effect is fundamentally due to the human chitin-binding domain.