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BACTERIAL CELLULOSE MODIFIED THROUGH RECOMBINANT PROTEINS AS A SCAFFOLD FOR NEURONAL CELL CULTURE

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INTRODUCTION

The challenge in nerve regeneration is to construct biological substitutes that when implanted at the injury site are capable to maintain a continuous path for regeneration and promoting the infiltration of cells to secrete inductive factors for axonal elongation (Prabhakaran, Venugopal et al. 2008). Biological scaffolds, composed of natural polymers combined with extracellular matrix molecules, have been shown to facilitate the constructive remodeling of several tissues by the establishment of an appropriated environment essential for the regulation of cell processes (Adams and Watt 1993; Badylak, Freytes et al. 2009). In recent years, bacterial cellulose (BC), a glucose polymer secreted by *G. xylinus* emerged as a promising biomaterial in tissue engineering due its properties such as high crystallinity, wettability, high tensile strength, moldability *in situ* and simple production (Svensson, Harrah et al. 2004). BC is produced in a pure form, free of other polymers (Jonas and Farah 1998; Vandamme, De Baets et al. 1998). These characteristics, beyond its biocompatibility, make the BC an ideal material for tissue engineering constructs.
The immobilization of proteins on biomaterials surface is a strategy to control and guide, with high selectivity, the interactions between cells and materials (Massia, Stark et al. 2000; Kam, Shain et al. 2002; Hersel, Dahmen et al. 2003). As an alternative to peptide chemical grafts, the use of recombinant proteins containing carbohydrates binding domains (CBMs), such as the CBM3, which has affinity by cellulose, fused to bioactive peptides represents an attractive way to specifically adsorb these peptides on cellulose surface (Wang, Wu et al. 2006; Andrade, Moreira et al. 2008). The main purpose of this work was to produce recombinant proteins containing a bioactive peptide fused to the CBM3 to functionalize BC surface in order to optimize material biocompatibility. Neuronal and mesenchymal cell adhesion and viability were evaluated on these modified surfaces. The neurotrophin expression by the mesenchymal cells on BC was also verified.

MATERIAL AND METHODS

Recombinant protein production

In this work, we produced 3 recombinant proteins: KHIFSDDSSE-LK-CBM3, IKVAV-LK-CBM3, and *ex*IKVAV-LK-CBM3 (CSRARKQAASIKVAVSADR-CBM3). Coding sequences were obtained by PCR using the pET21a-CBM3 vector. The PCR products were purified, digested with restriction enzymes, and cloned into the expression vector pET21a. The *E. coli* XL1 Blue was used as cloning strain and the integrity of cloned PCR products was verified by DNA sequencing. The recombinant proteins were purified by affinity chromatography.

Cell culture

The cell culture on the BC membranes was performed as follows: the proteins were added to a 24-well polystyrene plate (0.25 mg protein per well) covered with the BC pellicles. The unbound proteins were removed and the BC pellicles washed with PBS. Cells were then seeded in serum-free medium (excepted SH-SY5Y cells) at a density of 6×10^4 cells/well on BC pellicles. After 2 h, the wells were washed with PBS and transferred to new wells where complete medium was added. The control used was the BC membranes treated only with buffer.
SH-SY5Y, N1E-115, HMEC-1, PC12, rat Mesenchymal stem cells (MSCs), and mice astrocytes were maintained under standard tissue culture condition and specific culture medium. Cell adhesion was determined by mitochondrial activity through a MTS colorimetric assay. The viability of the cells cultured on BC membranes coated with the recombinant proteins was determined through the live/dead assay. The expression of nerve growth factor (NGF) neurotrophin by the MSCs was measured using a commercial ELISA kit.

RESULTS

The recombinant proteins IKVAV-CBM3, *ex*IKVAV-CBM3 and KHIFSDDSSE-CBM3, were successfully expressed in *E. coli*, purified and stably adsorbed to the BC membranes. The results showed that the *ex*IKVAV-CBM3 was able to improve the adhesion of both neuronal and mesenchymal cells (MSC), while IKVAV-CBM3 and KHIFSDDSSE-CBM3 presented only a slight effect on mesenchymal cell adhesion, and no effect on the other cells (Fig 1).

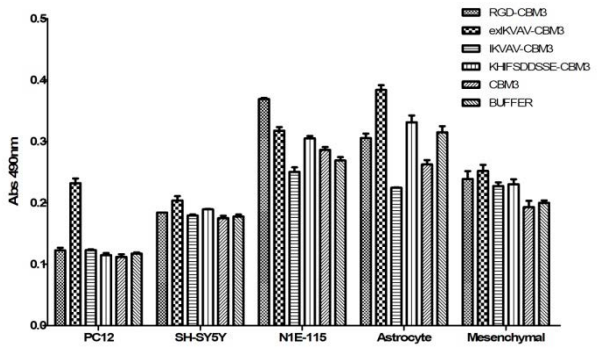

Figure 1 MTS assays cells seeded on BC pellicles coated with recombinant proteins. Cells were able to adhere in BC surfaces for 2h. The control was BC pellicle treated with Buffer without recombinant proteins.

Also, after 2 weeks in culture PC12 and mesenchymal cells on BC membranes coated with the recombinant protein *ex*IKVAV-CBM3 remained adhered and alive and control wells had fewer cells attached, mainly in PC12 culture (Fig 2).

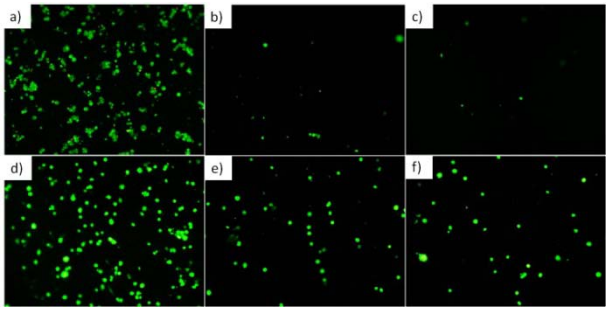

Figure 2 Images showing the live/dead assay of PC12 (a,b,c) and mesenchymal (d, e, f) cells cultured for 2 weeks on BC coated with *ex*IKVAV-CBM3 (a and d); CBM3(b and e); Buffer (c and f).

The MSCs expressed and released NGF when adhered on the BC membranes, creating a microenvironment that promotes neuronal regeneration.

CONCLUSIONS

The recombinant peptides were successfully expressed in *E. coli* and adsorbed in a stable way to the cellulose membranes. The recombinant protein *ex*IKVAV-CBM3 strongly improved PC12 and mesenchymal cell adhesion, indicating that this recombinant protein can be used in BC scaffolds for neural tissue engineering applications.

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