OMICS International

5th World Congress on Materials Science & Engineering

June 13-15, 2016 Alicante, Spain

Reduced graphene oxide absorbed on silk fibroin electrospun mats enhances neurite differentiation of PC-12 cells

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Consejería de Agricultura y Agua

Introduction

Silk fibroin and graphene are both promising biomaterials. Hybrid scaffolds combining their properties could be attractive for tissue engineering applications. Specifically, reduced graphene oxide (rGO) is biocompatible and electrical conductor, which is an interesting property for nervous tissue regeneration. PC-12 cells are a model cell system for a variety of neural functions as they can further differentiate into nerve cells when stimulated by nerve growth factor (NGF).

The aim of this assay was to induce neurite formation on *Bombyx mori* silk fibroin (SF) based biomaterials and in combination with rGO.

Materials & Methods

Cocoons of B. mori were degummed and SF was dissolved and concentrated by dialysis until a final 18 % (w/v) dissolution to be employed for the electrospinning (Figure 1). The electrospinning conditions were adjusted so that the Taylor cone was stable and the electrospun mats were annealed by immersion in methanol. SF mats were coated by absorption of GO in aqueous suspension (1 mg/mL) and followed a GO reduction by incubation of the mats with ascorbic acid 20mM during 3 h at 70ºC (SF+rGO mats).

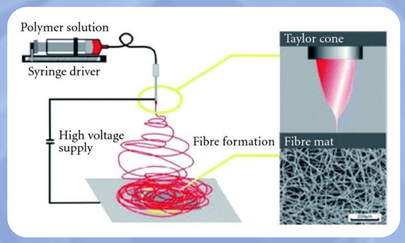

Figure 1. Electrospining process with a detail of the Taylor cone

PC-12 cell line was seeded on the electrospun mats at a density of 10000 cells/cm^2 in RPMI 1640 expansion media (10% horse serum, 5% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin). For cell differentiation, complete expansion media were substituted 3 days after seeding by RPMI 1640 differentiation media (2% horse serum, 1% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin), being this differentiation media carefully replaced each 24 h until the end of the study. In the case of SF+rGO+NGF mats, 50 ng/mL of NGF were also added to the differentiation media each time (positive control).

7 days after seeding, cells were fixed using 4% parafomaldehyde and stained with a dual-color Neurite Outgrowth Staining Kit (Molecular probes, Carlsbad, CA, USA) to visualize and quantify relative neurite differentiation (bright orange staining) and cell viability (green fluorescence) by image analysis using ImageJ. Scanning electron microscopy (SEM) was used to visualize the adhesion, growth and morphology of cells seeded on the mats.

Results

Cells growing on SF mats showed no differentiation (0%), nevertheless, PC-12 cells on SF+rGO mats showed a statistically similar neurite differentiation to that obtained with NGF stimulation (Figure 2). Although, neurite length of cells growing on SF+rGO mats was lower than those stimulated with NGF (Figure 3).

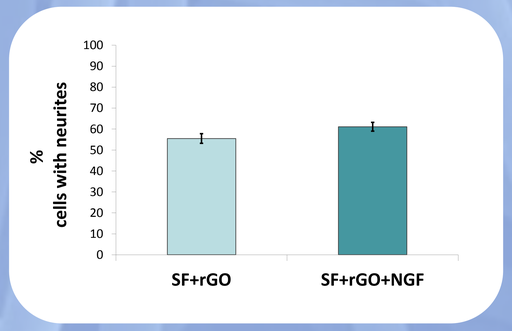

Figure 2. Percentage of differentiated PC-12 cells growing on electrospun mats

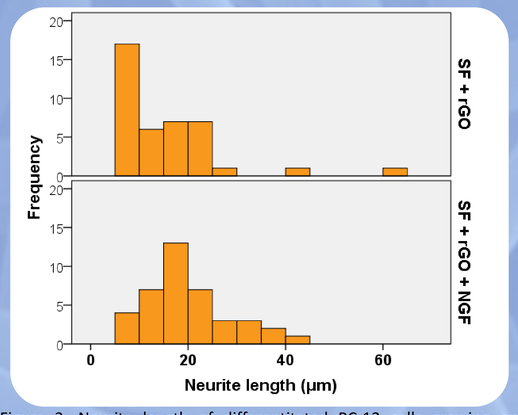

Figure 3. Neurite length of differentitated PC-12 cells growing on electrospun mats coated with rGO with and without NGF stimulation

Micrographs of PC-12 growing on electrospun mats showed an excellent cell adhesion in all the SF+rGO mats, the cells appeared well spread with thin filopodia and establishing a continuous monolayer of cells, denoting an optimal proliferation rate (Figure 4). Nevertheless, PC-12 seeded on SF mats showed worse adhesion and expansion, appearing rounded, in cluster-like groups and without filopodia (images not shown).

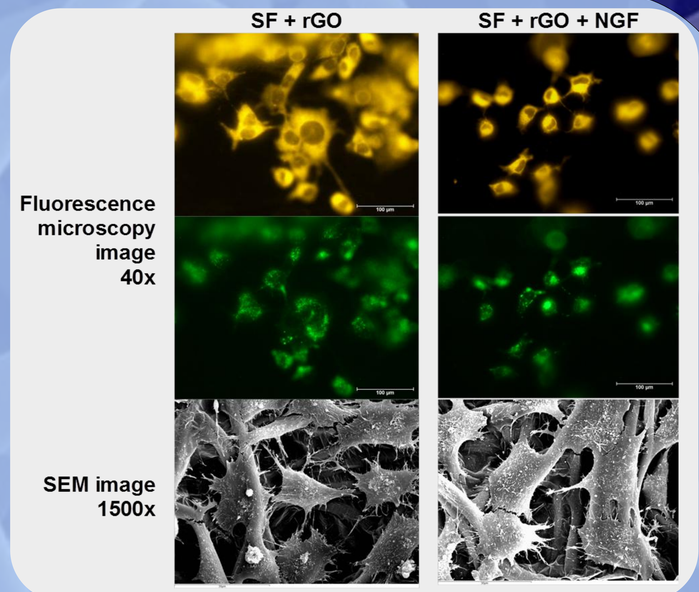

Figure 4. Micrographs of PC-12 growing on electrospun mats at different magnifications from optical fluorescent microscope and SEM

Viability was also appraised above 90% by the cell-permeable fluorescent dye, obtaining the same good viability values from the cells growing on SF mats.

Conclusion

rGO coating of SF electrospun mats induce per se neurite differentiation of PC12 cells, similar to neurite differentiation under stimulation with NGF, while nude SF mats induce no cell differentiation. Thus, the combination of SF with rGO seems to be an interesting biomaterial for nervous tissue engineering.

Acknowledgements

The authors acknowledge financial support from Spanish Government (MCI) Project MAT2011-24973 and partial support (80%) of the ERDF/FEDER Operative Program of the Region of Murcia 2007–2013, from the UE.