# Electrical stimulation of PC-12 cells cultured on silk fibroin scaffolds coated with reduced graphene

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## Abstract

New approaches to neural research require biocompatible materials capable to act as electrode structures or scaffolds in order to stimulate or restore the functionality of damaged tissues.

Graphene is a conducting material introduced in the field of tissue engineering due to its good biocompatibility and potential applications in biomedicine [1]. Silk fibroin (SF) is also a well-known biocompatible material in itself that combines with graphene producing hybrid films formats [2-4], providing an excellent support for cell proliferation [5]. However, the use of electrospun mats seems to be a better choice due to the biomimetic configuration with an extracellular matrix. Therefore, the approach proposed in the present work explores the combination of reduced graphene oxide (rGO) adsorbed on SF mats in order to confer them electroconductive properties [5].

PC-12 cell line was chosen for the study since these cells can be differentiated into a neuronal-like phenotype by exposing to NGF. The differentiation levels achieved with this treatment (SF/rGO/NGF) were compared (Fig.1) to the ones obtained in cells growing on: pure SF mats (SF), mats coated with rGO submitted to electrical stimulation (SF/rGO/ES) and mats coated with rGO without anyother stimulus (SF/rGO).

The method of production of these scaffolds barely alters the mechanical properties of pure SF mats. However, multiple benefits are obtained by means of the coating with rGO. In addition to the optimal viability detected in cells growing on all the produced materials, a clear improvement of adhesion and proliferation is exhibited in mats containing rGO. The stimulus provided by the rGO itself induces a significant differentiation level to neuronal-like phenotypes. However, the percentage of differentiation can be increased by means of the application of ES (100mV during 2h) or the treatment with NGF, being the neurite outgrowth more pronounced when electric currents are applied to the cell cultures.

## Image

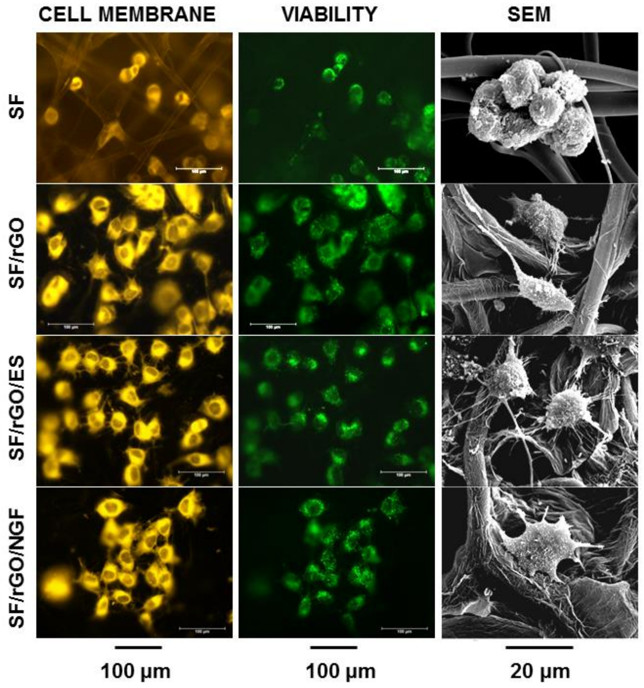


**Figure 1.** Micrographs of PC-12 cells growing on the studied electrospun materials at 10 days after the seeding. Fluorescence images of cell membrane and cell viability staining respectively (40X). Representative SEM micrographs (2000X). "SF" refers to the uncoated materials made of fibroin; "SF/rGO" are the rGO coated materials without any other stimulus than the presence of rGO in their composition; "SF/rGO/ES" refers to the rGO coated mats submitted to the treatment of electrical stimulation; "SF/rGO/NGF" are these materials coated with rGO were the neuritogenesis was stimulated by means of the treatment with NGF and electrical stimulation was not applied.

## Recent Publications


1. Rodríguez-.Lozano, F.J., García-Bernal, D., Aznar-Cervantes, S.D., Oñate-Sánchez, R.E., Moraleda, J.M. (2015) Potential of Graphene for Tissue Engineering Applications. Translational Research, 166(4):399-400.
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4. Jose G. Martinez, Salvador Aznar-Cervantes, Jose L. Cenis, Toribio F. Otero. (2016) Graphene adsorbed on silk-fibroin meshes: Biomimetics and reversible conformational movements driven by reactions. Electrochimica Acta, 209: 521–528.
5. [33]F.J. Rodríguez-Lozano, D. García-Bernal, S. Aznar-Cervantes, M.A. Ros-Roca, M.C. Algueró, N.M. Atucha, A.A. Lozano-García, J.M. Moraleda, J.L. Cenis, Effects of composite films of silk fibroin and graphene oxide on the proliferation, cell viability and mesenchymal phenotype of periodontal ligament stem cells, J. Mater. Sci. Mater. Med. 25 (2014) 2731–2741.


## Biography

Dr. Salvador D. Aznar Cervantes, born on 22 January 1983, works as a researcher in the Department of Biotechnology, in the R&D Center in Biotechnology and Biomedicine, IMIDA (Murcia). He obtained his degree in Biology from the University of Murcia (2006), then he completed his doctoral thesis, working as a grant holder (FPI-INIA), under the direction of Dr. José Luis Cenis Anadón, in January 2013. During the development of his PhD, he researched on biotechnological and biomedical applications of the silk worm (Bombyx mori). This period was complemented with 3 successive visits (2010, 2011, 2012) to the Department of Chemical Engineering of Massachusetts Institute of Technology (MIT), where he also collaborated with Tufts University (Professor David L. Kaplan) and the Massachusetts General Hospital (Professor Robert Redmond).

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**Notes/Comments:**


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